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37 No. 4

Journal of DENTAL RESEARCH

Founded by William J. Gies in 1919

OFFICIAL PUBLICATION OF THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Editor
FRANK J. ORLAND

Published for
THE INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

By
THE C. V. MOSBY COMPANY
ST. LOUIS 3, MO.

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Vol. 37, No. 4, August, 1958. Journal of Dental Research is published bi-monthly by The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, Mo. Subscription rates: United States and its Possessions $10.00, Students $5.00; Canada, Latin-America, and Spain $10.50, Students $5.50; Other Countries $11.00, Students $6.00. Single copies $2.50 postpaid. Entered as second-class matter June 24, 1919, at the Post Office at St. Louis, Mo., under the Act of March 3, 1879. Printed in the U. S. A.

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Organized: New York City, Dec. 10, 1920. Of those at the meeting of organization, the following remain active members: Adolph Berger, Theodor Blum, G. W. Clapp, H. S. Dunning, A. H. Merritt, B. B. Palmer, L. M. Waugh.

Objects (Const., Art. II): The association has been established to promote broadly the advancement of research in all branches of dental science and in the related phases of the sciences that contribute directly to the development of oral health service, and as a whole; and further, to encourage and facilitate cooperative effort and achievement by, and mutual helpfulness among, investigators in all nations in every division of stomatology to the end that dentistry may render cumulatively more perfect service to humanity.

Membership: total (March, 1957), 916. *Eligibility (Const., Art. III, Sec. 1).*—"Any person who has conducted, and published an account of, a meritorious original investigation in dental science, or in any of the sciences contributory to oral health, shall be eligible to membership in this Association, providing such person conforms to the recognized standards of professional ethics." *Election.*—New members may be elected *only at general meetings of the Association.* Membership in a section is secondary to membership in the Association as a whole. Removal of members from one geographic center to another does not affect their membership in the Association.

Endowment fund of the International Association for Dental Research (as of March, 1956): $1,802.34. There have been no expenditures from this fund.

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EDITORIAL VIEWS AND NEWS

In the last issue of the JOURNAL, it was mentioned that a questionnaire would be sent to all I. A. D. R. members by the Secretary-Treasurer in order to gain a certain cross-sectional consensus regarding future editorial policies. The response has been somewhat gratifying insofar as 315 forms have been returned to date from among more than 900 actually mailed out. This is considered a good return by public opinion "pollsters." But quite aside from mere quantity, the qualitative aspects of the comments contained were even more commendable. A great number (361) of constructive suggestions were made, all of which will be considered by the Editor and his Staff.

Questionnaire Results.—Specifically, the expression of opinion was as follows:

To the first question regarding the new Section in the JOURNAL (consisting of short, rapidly published notes) the answers were 242 in favor, 49 against, 19 undecided, and 83 made various comments. Thus, what apparently was originally a good idea, and is now supported by the vast majority of those replying, will go into effect with the October issue. Hence any such research notes received currently by the Editorial Office will be published in the next issue.

On the matter of whether there should appear on the first page of each published regular article, a concise, informative abstract instead of a terminal summary, there were 246 in favor, 48 against, 21 undecided with 80 writing in comments. Whether biographical sketches of I. A. D. R. members should be published in the JOURNAL (as in the October 1952 issue) brought forth a variety of opinions: 137 favorable, 113 against, 59 undecided, and 103 prepared comments. Since there are more difficulties encountered in carrying out these latter two thoughts and since many of the readers indicated they felt the I. A. D. R. needed a biographical sketch, but *not* published in the JOURNAL, the Publication Committee will need to take these opinions and comments under consideration. If there is a strong motivation for a biographical directory, perhaps the Archives Committee could go into action on such a project, listing in detail only those members not already named in "American Men of Science." (It also is interesting to note 282 signed their names to the questionnaire although this was not necessary as stated on the original form.)

Sectional Officers.—Another effort has been made to bring up to date the list of I. A. D. R. Sectional Officers as they appear on the inside area of the front cover of the JOURNAL. Most of the Secretaries contacted, including those abroad, have responded to provide the Editor with their current officers, including the Sectional President or Chairman, who is now listed in a new column. Nevertheless, new additions will need to be made to keep the published list current and correct. It may be of interest to observe that three Secretaries of Sections are women, all active in research: Lisbeth Baumann, Mary C. Crowley, and Verda Elizabeth James. We should encourage women as workers in oral research!

The JOURNAL OF DENTAL RESEARCH, as most other journals, must make use of every square centimeter of space, including the JOURNAL covers. Thus, when readers have their copies bound by volumes it might be well to insist that the covers of each number be kept intact since bookbinders invariably will otherwise discard them, as has happened to some of the early bound volumes even here at the Zoller Memorial Dental Clinic.

That Opening Sentence.—Everyone is aware or should be aware of the difficulties encountered in preparing an opening sentence for a manuscript. Recently Gerald J. Cox (Director of Research at the School of Dentistry, University of Pittsburgh) showed me a concise original essay which he wrote on "The Opening Sentence." I considered it edifying enough to ask Dr. Cox's permission to reproduce it under EDITORIAL VIEWS AND NEWS in the October issue of the JOURNAL.

—FRANK J. ORLAND, D.D.S., PH.D.
EDITOR

THE EFFECT OF SUGAR IN VARIOUS FORMS ON THE RATE OF SPREAD OF OCCLUSAL CARIES IN THE LOWER MOLARS OF RATS

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INTRODUCTION

THE results of the Vipeholm Study[1] suggested that sugar in solution is less cariogenic than sugar in a solid sticky form. Although this effect was apparent in relation to the initiation of dental caries, no clear deductions could be made concerning the effect of sugar in solution on the rate of spread of lesions already established. This experiment was designed to study this latter problem.

METHODS

General.—

For descriptive convenience, the experiment can be divided into two periods. During the first period all the animals were given the same cariogenic diet and water. This period will be called the "caries-initiation" period.

As soon as occlusal caries was detected in a lower molar of a given rat, the rat was assigned to 1 of 6 groups. Some of the rats were assigned to a sacrifice control group and were killed immediately. The other rats were assigned to 5 other groups. The rats in each of these groups were given a different diet to determine the effect of the different feeding regimens on the spread of caries. This period will be called the "spread-of-caries" period.

Caries-Initiation Period.—Osborne-Mendel strain rats aged 20 to 21 days were fed ad libitum with distilled water and Diet 580. The latter is a cariogenic solid diet devised by Stephan and Harris.[2] It consists of fine granulated sugar, 66 parts; dried skim milk powder, 32 parts; and desiccated liver, 2 parts. Diet 580 was placed in metal-capped feeding cups and distilled water was placed in 150 ml. Pyrex glass beakers which were wired to the front of the cages. Both Diet 580 and the water were renewed every 2 days. The amounts of each that were consumed by each rat were calculated from the difference in weights at the beginning and end of each 2-day period. All rats were housed in individual cages and weighed at weekly intervals.

Each rat was examined at weekly intervals until initial caries was detected in an occlusal fissure of a mandibular first or second molar. Standardized probes for the clinical detection of caries were made from S.S.W. No. 42 fine smooth

Supported by a grant from American Bottlers of Carbonated Beverages.
Received for publication May 19, 1957; revised by authors April 18, 1958.

broaches. All examinations were made under a 20× binocular dissecting microscope. Johansen's Immobilizer[3] was used to hold each animal during the clinical examination. Initial examinations were made on unanesthetized animals. However, when the animals weighed more than 100 grams they were anesthetized by the intraperitoneal injection of a solution of Nembutal.

When initial caries was detected, the location and size of each lesion were recorded and the rat was assigned to 1 of the 5 groups for the spread-of-caries period of the experiment. Rats assigned to the Sacrifice Control Group were killed immediately and their dry skulls were examined as a check on the accuracy of the clinical examination.

Spread-of-Caries Period.—In assigning animals to the various groups in this part of the experiment an attempt was made to equalize each group in terms of the following criteria: litter, sex, age when caries was detected, number of lesions, previous food consumption, and weight.

The 5 groups in this phase of the experiment have been named according to the diet they were given as follows: (1) 580 plus Water, (2) 580M plus Water, (3) 580M plus 10 per cent Sucrose, (4) 580M plus Carbonated Beverage, and (5) 580M plus Orange Juice.

Diet 580 was the same as that used in the caries-initiation period. Diet 580M contained skim milk powder and desiccated liver only, and was made up by adding 2 grams of desiccated liver to each 32 grams of skim milk powder. Sucrose solution was a 10 per cent solution of sucrose in water. The carbonated beverage used was a popular brand containing 9.7 per cent carbohydrate and citric acid for acidulation. The orange juice solution was made by diluting frozen concentrated orange juice to give a final carbohydrate content of 10 per cent.

Each animal assigned to Groups 580M plus Water, 580M plus 10 per cent Sucrose Solution, 580M plus Carbonated Beverage, and 580M plus Orange Juice was paired for feeding purposes on the basis of age, sex, weight, and previous food consumption with 1 animal in Group 580 plus Water. Animals in Group 580 plus Water were given a daily ration of either 12 or 15 grams of Diet 580, and water was given ad libitum. Then, except for Group 580M plus Water, the amount of solid diet 580 M, and the amounts of sucrose solution, carbonated beverage, or orange juice given were adjusted so that equivalent amounts of sugar (solid or liquid), milk powder, and desiccated liver were consumed by the paired animals in each group.

The weights of solid and liquid actually made available to the various groups are shown in Table I. Since all the animals did not consume their entire ration each day, only the actual amounts consumed were weighed and recorded.

The animals were kept on this regimen for 19 to 22 days. The date of initial assignment to groups varied from one animal to another, since all the animals did not develop a clinically detectable occlusal lesion on the same day. However, after caries was detected, all paired animals were kept on the experimental regimen for the same length of time.

TABLE I

WEIGHT IN GRAMS OF SOLID AND LIQUID DIET MADE AVAILABLE TO RATS DURING THE SPREAD-OF-CARIES PERIOD

	GROUP									
	580 PLUS WATER		580M + WATER		580M + SUCROSE SOLUTION		580M + CARBONATED BEVERAGE		580M + ORANGE JUICE	
SUBGROUP	DIET 580	WATER	DIET 580M	WATER	DIET 580M	10 PER CENT SUCROSE SOLUTION	DIET 580M	CARBONATED BEVERAGE	DIET 580M	ORANGE JUICE
i.	12	ad. lib.	ad. lib.	ad. lib.	4	79	4	82	4	79
ii.	15	ad. lib.	ad. lib.	ad. lib.	5	99	5	102	5	99

At the end of the experimental period, the animals were anesthetized and the occlusal surfaces of all lower molars were re-examined under the same conditions as at the end of the caries-initiation period. In addition, to prevent examination bias, the identification labels on each cage were reversed, the cages were shifted around on the rack and renumbered by an independent person. Thus, at the time of the examination, the examiner (G. N. D.) knew neither the true number of the animal nor the group to which it belonged.

After the final clinical examination, each animal was sacrificed by administering an overdose of Nembutal.

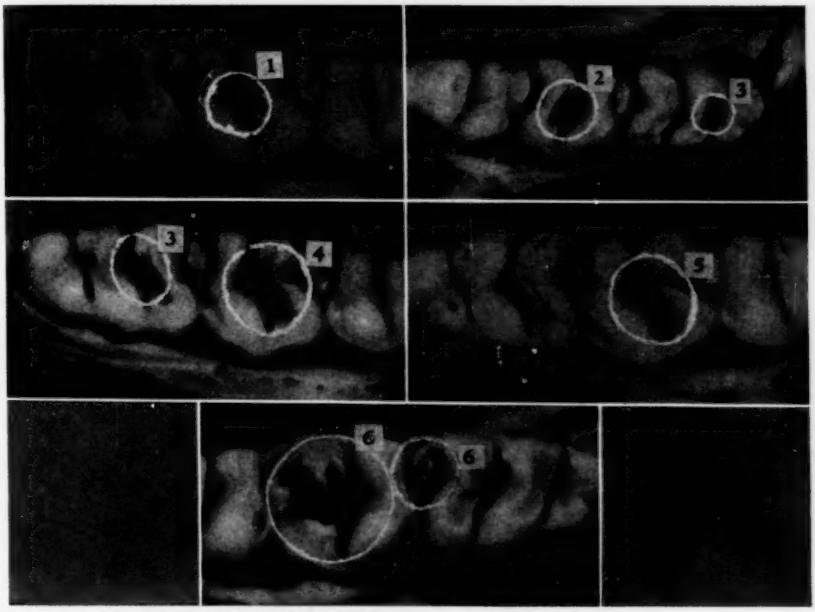

Fig. 1.—Photographs of cavities scored as 1 to 6.

Scoring System.—

The location of each lesion was drawn on an anatomic representation of the appropriate tooth. The size of each lesion was given a numerical value as follows:

1—Small, definitely softened region in enamel only.
2—More widespread softening of enamel with slight penetration into dentin.
3—Deep penetration of dentin with slight undermining of a cusp or marginal ridge.
4—Widespread decay of enamel and dentin with undermining and destruction of approximately half a cusp or marginal ridge.
5—Widespread decay of enamel and dentin with undermining and destruction of more than half a cusp or marginal ridge.
6—Complete destruction of a cusp or marginal ridge.

TABLE II

FOOD AND WATER CONSUMPTION IN GRAMS DURING THE CARIES-INITIATION PERIOD

GROUP TO WHICH ANIMALS WERE ASSIGNED WHEN CARIES WAS DETECTED	NUMBER OF ANIMALS			AVERAGE NUMBER OF DAYS ON CARIOGENIC DIET			AVERAGE DAILY FOOD CONSUMPTION IN GRAMS											
							DIET 580									WATER		
							SUGAR			MILK POWDER			DESICCATED LIVER					
	♂	♀	TOTAL	♂	♀	COMBINED SEXES	♂	♀	COMBINED SEXES	♂	♀	COMBINED SEXES	♂	♀	COMBINED SEXES	♂	♀	COMBINED SEXES
Sacrifice	8	8	16	38.1	38.9	38.5	7.7	7.4	7.6	3.7	3.6	3.7	0.2	0.2	0.2	15.2	17.8	16.5
Diet 580 + water	8	8	16	40.1	40.7	40.4	8.2	8.1	8.2	4.0	3.9	4.0	0.2	0.2	0.2	16.6	16.6	16.6
Diet 580M + water	7	7	14	42.7	38.7	40.7	8.2	7.8	8.0	4.0	3.8	3.9	0.2	0.2	0.2	16.3	17.7	16.8
Diet 580M + 10 per cent sucrose	6	9	15	43.0	41.9	42.3	8.5	7.7	8.0	4.1	3.7	3.9	0.3	0.2	0.2	16.8	16.9	16.9
Diet 580M + carbonated beverage	8	7	15	43.0	40.6	41.9	8.7	7.2	8.0	4.2	3.5	3.9	0.3	0.2	0.2	17.3	16.6	16.9
Diet 580M + orange juice	7	8	15	39.7	41.4	40.6	8.3	7.6	7.9	4.0	3.7	3.8	0.2	0.2	0.2	16.2	17.0	16.6
Totals	44	47	91															

Using this system one can calculate (a) the total occlusal caries score per animal, by adding the scores of each lesion; (b) the average occlusal caries score per animal, by dividing the total occlusal score per animal by the number of occlusal lesions; (c) the total occlusal score increase in a particular group during the spread-of-caries period, by subtracting the total occlusal score for the group at the beginning of the period from the total occlusal score for the group at the end of the period.

RESULTS

Caries-Initiation Period.—Ninety-one animals (44 male and 47 female) were used in this part of the study. Details of the "food consumption period" are presented in Table II.

These data are presented to show the extent to which the groups to which the animals were assigned for the spread-of-caries period were equalized in terms of food consumption, the number of males and females, and the number of days which were required for the initiation of occlusal caries.

Compared with females, the males consumed slightly more of the solid diet each day and slightly less water. The average daily consumption of Diet 580 was 12.6 grams for males and 11.6 grams for females. The average daily consumption of water was 16.4 grams for males and 17.1 grams for females.

At the end of the caries-initiation period, 181 occlusal lesions were diagnosed—79 in 44 males, and 102 in 47 females. The average length of time for occlusal caries to be initiated was 41.0 days (range: 26-61 days) for males and 40.4 days (range: 26-65 days) for females.

Data on the number of occlusal lesions and the occlusal caries scores have been summarized in Table III. These data show the extent to which the groups organized for the spread-of-caries period were equalized in terms of the number and size of the occlusal lesions which were diagnosed at the end of the caries-initiation period. All groups were similar in the number of occlusal lesions per rat. The average caries score per rat was also similar in all groups except that in the 580 plus Water group the average caries score per rat was lower than in the other groups.

Spread-of-Caries Period.—Sixteen of the animals were sacrificed at the end of the caries-initiation period so that 75 animals (36 males and 39 females) were used in the spread-of-caries period.

The data on the food and liquid consumption during the spread-of-caries period are shown in Table IV. The amount of sucrose consumed in solid form by the animals in Group 580 plus Water was similar to the amount of sucrose consumed in liquid form by the animals in Groups 580M plus 10 per cent Sucrose and 580M plus Carbonated Beverage. The animals in Group 580M plus Orange Juice failed to consume their full daily ration of orange juice, consequently their intake of sucrose in solution was lower than that of the animals in the other 3 groups.

The animals in Group 580M plus Water (which were given Diet 580M and water ad libitum) consumed approximately three times as much milk powder

TABLE III

NUMBER OF OCCLUSAL LESIONS, AND OCCLUSAL CARIES SCORES AT THE END OF THE CARIES-INITIATION PERIOD

GROUP TO WHICH ANIMALS WERE ASSIGNED WHEN CARIES WAS DETECTED	NUMBER OF RATS			NUMBER OF OCCLUSAL LESIONS DIAGNOSED			AVERAGE NUMBER OF LESIONS PER RAT						CARIES SCORE PER RAT					
							♂		♀		COMBINED SEXES		♂		♀		COMBINED SEXES	
	♂	♀	TOTAL	♂	♀	TOTAL	MEAN	S.D.*	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
Sacrifice	8	8	16	19	13	32	2.4	1.4	1.6	1.1	2.0	1.1	3.5	1.9	3.1	1.6	3.3	1.5
580 + water	8	8	16	12	16	28	1.5	1.3	2.0	1.3	1.8	1.2	2.0	1.3	2.9	2.3	2.5	1.9
580M + water	7	7	14	10	20	30	1.4	0.8	2.8	2.2	2.1	1.6	2.4	1.9	4.7	3.9	3.6	2.9
580M + 10 per cent sucrose	6	9	15	10	19	29	1.7	1.0	2.1	1.5	1.9	1.6	3.0	2.1	3.4	2.9	3.3	2.3
580M + carbonated beverage	8	7	15	16	15	31	2.0	1.3	2.1	1.2	2.1	1.0	3.6	2.7	3.8	2.6	3.7	2.3
580M + orange juice	7	8	15	12	19	31	1.7	0.9	2.4	1.5	2.1	1.0	3.6	2.6	3.9	2.4	3.7	2.3
Totals	44	47	91	79	102	181												

*S.D. = Standard deviation.

TABLE IV

FOOD AND LIQUID CONSUMPTION AND WEIGHT GAINS DURING THE SPREAD-OF-CARIES PERIOD

DIETARY GROUP	AVERAGE NUMBER OF DAYS ON REGIME			NUMBER OF ANIMALS			AVERAGE DAILY CONSUMPTION IN GRAMS															AVERAGE DAILY WEIGHT GAIN IN GRAMS		
							SOLID SUGAR			SUGAR IN SOLUTION			MILK POWDER			LIVER EXTRACT			WATER					
	♂	♀	AVERAGE	♂	♀	TOTAL	♂	♀	AVERAGE	♂	♀	AVERAGE	♂	♀	AVERAGE	♂	♀	AVERAGE	♂	♀	AVERAGE	♂	♀	AVERAGE
580M + water	19.8	20.5	20.2	8	8	16	8.9	8.1	8.5				4.3	4.0	4.1	0.3	0.2	0.3	21.8	21.1	21.4	1.97	1.43	1.7
580M + water	21.0	20.0	20.5	7	7	14							11.8	11.1	11.5	0.8	0.7	0.7	43.0	40.1	41.6	1.9	1.5	1.7
580M + 10 per cent sucrose	20.5	20.5	20.5	6	9	15				8.3	8.7	8.6	4.1	3.8	3.9	0.3	0.2	0.3	74.8	78.8	77.3	1.3	1.2	1.2
580M + carbonated beverage	21.1	20.3	20.7	8	7	15				8.7	8.1	8.4	4.4	3.3	3.9	0.3	0.2	0.3	80.9	75.4	78.5	1.3	1.3	1.3
580M + orange juice	20.4	20.6	20.5	7	8	15				6.4	5.1	5.7	4.3	4.1	4.2	0.3	0.3	0.3	57.8	45.9	51.5	1.1	1.6	1.4

TABLE V

INCREASE IN SIZE OF OCCLUSAL LESIONS DETECTED BY CLINICAL EXAMINATION AT THE BEGINNING AND END OF THE SPREAD-OF-CARIES PERIOD

DIETARY GROUP	NUMBER OF ANIMALS	NUMBER OF OCCLUSAL LESIONS	TOTAL OCCLUSAL CARIES SCORES AT THE:		TOTAL SCORE INCREASE	MEAN SCORE INCREASE PER LESION	DIFFERENCE OF MEANS COMPARED WITH GROUP 580 PLUS WATER	"t"	p
			BEGINNING	END					
580 + water	16	22	31	63	42	1.91	–		
580M + water	14	24	41	75	34	1.42	0.49	2.88	0.01
580M + sucrose solution	15	23	41	80	39	1.69	0.22	1.29	N.S.
580M + carbonated beverage	15	25	50	88	38	1.52	0.39	2.35	0.05
580M + orange juice	15	24	49	75	26	1.08	0.83	5.55	0.001
Totals	75	118							

and desiccated liver as the animals in the other groups and drank approximately twice as much distilled water as the animals in Group 580 plus Water.

The animals in Groups 580 plus Water, 580M plus 10 per cent Sucrose, 580M plus Carbonated Beverage, and 580M plus Orange Juice consumed similar quantities of milk powder and desiccated liver.

The animals in Group 580 plus Water consumed less water than did the animals in Groups 580M plus 10 per cent Sucrose, 580M plus Carbonated Beverage and 580M plus Orange Juice. This was expected since the animals in 580 plus water were given sugar in their solid diet and drank distilled water, whereas the animals in the other groups consumed their sugar and water in the form of 10 per cent sucrose solution, carbonated beverage, or orange juice, respectively.

The average daily gains in weight were similar in Groups 580M plus 10 per cent Sucrose, 580M plus Carbonated Beverage, and 580M plus Orange Juice, but were somewhat less than in Groups 580 plus Water and 580M plus Water.

Table V contains data relating to the increase in size of the occlusal lesions during the spread-of-caries period. Two things should be noted in interpreting these data. First, the results for males and females have been combined. Second, the data are confined to those lesions which were diagnosed both at the beginning and end of the experimental period. From Table III it will be seen that 181 occlusal lesions were diagnosed at the end of the caries-initiation period. Since 32 of these lesions were detected in the animals which were sacrificed at the end of the caries-initiation period the number of lesions which should have been under observation during the spread-of-caries period was 149. However, at the final clinical examination at the end of the spread-of-caries period only 118 of these lesions were detected. The data in Table V refer only to the 118 lesions.

These results show that the rate of spread of established occlusal caries was: (1) similar in Groups 580 plus Water, and 580M plus 10 per cent Sucrose Solution; (2) significantly lower in Groups 580M plus Water, 580M plus Carbonated Beverage and 580M plus Orange Juice, than in Group 580 plus Water; and (3) significantly lower in Group 580M plus Orange Juice than in all other groups including 580M plus Water.

DISCUSSION

Our results are based entirely on lesions which were diagnosed at both the initial and final examinations. This was done because, in spite of the usefulness of the Johansen Immobilizer for detecting caries in living rats, reversals in diagnosis occurred with approximately 20 per cent of minute lesions which involved enamel only (Score 1).

Our results show that sucrose in aqueous solution had about the same effect as solid sucrose on the spread of established caries, but the same intake of sugar in a carbonated beverage produced less expansion of caries, and a smaller intake of sugar in the Orange Juice Group produced even less expansion of caries. The results with orange juice can reasonably be attributed to the lower

intake of sucrose resulting from the refusal of the rats to consume as much of the juice as they did of carbonated beverage or aqueous sucrose solution. Actually, since the Anthrone method,[4, 5] which was used to measure the carbohydrate content of the orange juice concentrate, gives a positive reaction with all monosaccharides, disaccharides, and even polysaccharides such as cellulose, the intake of sucrose in the Orange Juice Group was probably less than shown in our figures (Table IV). Even with such reservations, the reduction in the spread of caries in the Orange Juice Group was greater than could be accounted for by the reduced intake of sucrose alone.

A limiting factor in the interpretation of our results is that the dried skim milk powder, which was the basis of the solid portion of the diet for all groups of animals, contained about 51 per cent of lactose.[6] Therefore, all the animals received lactose in addition to their rationed intake of sucrose. Nevertheless, because this lactose supplement was approximately the same in all groups, except the 580M plus Water Group, comparisons between the other groups are permissible. As far as the excepted group (580M plus Water) is concerned, it seems reasonable to suggest that the average daily intake of more than 5 grams of lactose might have been responsible for the spread of caries in this group.

SUMMARY

Osborne-Mendel strain rats were placed on a cariogenic diet until dental caries was detected in the occlusal surfaces of lower first or second molars. They were then assigned to different groups for a specified length of time to determine the effect of sucrose in various forms on the rate of spread of established lesions.

The results suggest that there is no difference between the effect on the spread of caries of sucrose in aqueous solution and sucrose in solid form. But when sucrose was fed in the form of a carbonated beverage and as a dilution of orange juice concentrate, dental caries progressed at a significantly slower rate.

The authors are grateful to Dr. Robert M. Stephan, National Institute of Dental Research, for valuable advice and for providing Osborne-Mendel strain breeding rats; also to Dr. Erling Johansen for invaluable suggestions, and for providing facilities at the School of Medicine and Dentistry, University of Rochester, N. Y.

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STUDIES OF THE COMPOSITION OF TEETH

VII. The Moisture Content of Calcified Tooth Tissues

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IN SPITE of the many studies of the composition of human calcified tissues, their moisture content has neither been investigated extensively nor established conclusively.[1-8] Usually investigations of the moisture content have been only incidental to the determination of the organic or inorganic composition of enamel and dentin. Furthermore, the criteria for what investigators have considered to be "dry" enamel and dentin is rather vague for, in dehydrating such tissues, the temperature, environmental moisture, and length of exposure to dehydrating agent have been quite variable in the different investigations. The establishment of criteria for an equable calcified tooth tissue in regard to moisture content is of considerable importance, however, in all types of analytical investigations directed toward the establishment of the protein content, heat labile components, and inorganic composition, for if the moisture content of the tissue being analyzed is allowed to vary, it is difficult to establish a base line for calculation of analytical data. Therefore, observations have been made of the moisture content of whole human teeth, and of separated and purified enamel and dentin under standardized conditions in an attempt to establish equable dehydrated calcified tooth tissues.

METHODS

Several approaches were made to the establishment of the moisture content of calcified tooth tissues. Initially, freshly extracted teeth were split and the pulp stripped from the pulp chamber. The moisture content of the split, pulpless teeth was then determined at varying humidities, atmospheric pressures, and temperatures. The moisture content was also determined for root dentin from freshly extracted teeth. Due to the technical difficulties of separating the tissues and at the same time maintaining their original moisture content, enamel from freshly extracted teeth could not be obtained for determination of moisture content.

In another approach to the determination of the moisture content and to the establishment of equable dry tissues, extracted human teeth were conditioned at 82° ± 3° F. and 36 to 49 per cent relative humidity for 14 days before determination of their moisture content. Enamel and dentin were prepared also by separating and purifying, according to the method described by Battistone and Burnett.[9] Enamel and dentin prepared by this method were 99.9

Received for publication July 6, 1957; revised by authors March 26, 1958.

per cent pure, and samples of each tissue were of 2 particle sizes: (1) particles which passed a 60-mesh screen but did not pass a 100-mesh screen (+60 –100 mesh particles) and (2) particles which passed a 100-mesh screen (100– particles). Samples of ground enamel and dentin were conditioned also for 14 days at 82° ± 3° F. and 36 to 49 per cent relative humidity. In another experiment, whole teeth and enamel and dentin were conditioned at 98° F. and 100 per cent relative humidity. After conditioning, the teeth and enamel and dentin were subjected to varying humidities, atmospheric pressures, and temperatures in order to determine moisture content under standardized conditions and to establish an equable tissue with regard to moisture content. Weight loss or gain was considered to indicate change in the moisture content of the tissues.

DEHYDRATION OF FRESHLY EXTRACTED WHOLE TEETH AND WHOLE DENTIN

Ten freshly extracted caries-free whole teeth, minus pulp, were obtained on a random basis without regard to tooth type, and dehydrated successively at 100° C. in vacuo and at 197° C. in vacuo. The moisture content of such teeth, as determined by dehydrating to a constant weight at 100° C. in vacuo, was 8.53 ± .33 per cent. The moisture content of the same teeth, as determined by dehydrating to a constant weight at 197° C. in vacuo, was 9.32 ± .37 per cent. The time required to establish a constant weight is shown in Table I for comparison with other rates of dehydration of whole teeth and tooth tissues. Approximately 24 hours were required to establish a constant weight of the freshly extracted whole teeth in vacuo at each temperature increment. The moisture content of dentin from 10 freshly extracted teeth was found to be 10.00 ± .55 per cent at 100° C. in vacuo, and 12.16 ± .80 per cent at 197° C. in vacuo. The time required to establish a constant weight for such dentin was 24 hours.

TABLE I

TIME IN HOURS REQUIRED TO ESTABLISH A CONSTANT WEIGHT FOR WHOLE TEETH AND ENAMEL AND DENTIN

	NUMBER OF TEETH OR TISSUE SAMPLES	100° C. ATMOSPHERIC PRESSURE	61° C. IN VACUO	100° C. IN VACUO	197° C.* IN VACUO
Freshly extracted whole teeth	10			23	23
Freshly extracted whole dentin	10			24	24
Conditioned whole teeth	10	168	24	26	24
Conditioned enamel	5	168		24	16
Conditioned dentin	5	442		32	40

*Indicates additional time required to establish a constant weight after initial dehydration at lower temperature.

DEHYDRATION OF CONDITIONED WHOLE TEETH AND TOOTH TISSUES

Whole teeth and samples of purified enamel and dentin conditioned at 82° ± 3° F. and 36 to 49 per cent relative humidity were brought to a constant

weight successively in normal atmosphere at 100° C., 61° C. in vacuo, 100° C. in vacuo, and at 197° C. in vacuo; conditioned enamel and dentin were brought to a constant weight at 100° C. in normal atmosphere and successively at 100° C. in vacuo and 197° C. in vacuo. The times required to establish constant weight at a given temperature and atmospheric condition are shown in Table I. At normal atmospheric pressure and 100° C., whole teeth and powdered enamel required 7 days to reach a constant weight, while the powdered dentin did not reach a constant weight for approximately 18 days. Conditioned whole teeth reached a constant weight at 61° C. in vacuo within 24 hours; enamel and dentin were not dehydrated at this temperature. At either 100° C. in vacuo or 197° C. in vacuo, 40 hours or less were required to establish constant weight for either conditioned whole teeth or conditioned powdered enamel and dentin. However, conditioned powdered dentin consistently required longer to reach a constant weight than did either conditioned powdered enamel or conditioned whole teeth.

The moisture lost by whole teeth and enamel and dentin conditioned at 82° ± 3° F. and 36 to 49 per cent relative humidity when subjected to varying temperatures and atmospheric conditions is shown in Table II. After a constant weight was established at each temperature increment and set of conditions, each sample was subjected to 82° ± 3° F. and 36 to 49 per cent relative humidity for a period of 24 hours before exposing it to a higher temperature and lower atmospheric pressure. In the case of conditioned whole teeth,

TABLE II

THE MEAN PERCENTAGE DEHYDRATION OF CONDITIONED WHOLE TEETH AND TOOTH TISSUES AT VARYING TEMPERATURES AND ATMOSPHERIC CONDITIONS*

TYPE OF TEETH OR TOOTH TISSUE	NUMBER OF TEETH OR TISSUE SAMPLES	100° C. ± 3° C. NORMAL ATMOSPHERIC PRESSURE	61° C. IN VACUO	100° C. IN VACUO	197° C. IN VACUO
Incisors and molars	10	6.2 ± .15	6.3 ± .26	6.5 ± .24	8.0 ± .24
Incisors	10	6.4 ± .16	6.5 ± .13	6.7 ± .46	8.3 ± .40
Molars	10	5.8 ± .03	6.0 ± .21	6.3 ± .24	7.6 ± .14
Enamel					
100- particles	5	0.45 ± .01		0.52 ± .04	0.81 ± .03
+60 -100 particles	5	0.17 ± .002		0.30 ± .003	0.39 ± .02
Dentin					
100- particles	5	6.3 ± .03		7.3 ± .17	8.7 ± .12
+60 -100 particles	5	6.1 ± .03		6.7 ± .04	8.2 ± .03

*Conditioned at 82° ± 3° F. and 36 to 49 per cent relative humidity; values expressed as mean and standard error.

approximately the same amount of moisture was removed when they were brought to a constant weight at 100° C. and normal atmospheric pressure as was removed at 61° C. in vacuo or 100° C. in vacuo. More moisture was lost, however, when the whole teeth were brought to a constant weight at 197° C. in vacuo. The moisture content of enamel was considerably less than that of whole teeth or powdered dentin. Particle size influenced the water content of powdered enamel, for there was less moisture in or on the conditioned enamel of larger particle size than in or on that of smaller particle size. The moisture

content of conditioned powdered dentin at the various temperatures and atmospheric conditions corresponded closely to that of conditioned whole teeth. Similar to whole teeth, subjecting dentin to 197° C. in vacuo removed more moisture than did drying such tissue at a lower temperature. In contrast to enamel, the particle size of dentin did not greatly influence its moisture content.

REHYDRATION OF TEETH DEHYDRATED AT 100° C. OR MORE

Three groups, each composed of 10 teeth, were dried at 100° C. and normal atmospheric conditions and three groups, each composed of 10 teeth, were dried at 197° C. in vacuo. As soon as a constant weight was obtained, the groups of teeth were returned to normal atmospheric pressure and 36 to 49 per cent relative humidity and maintained at 82° ± 3° F. for varying periods of time up to 28 days. The degree of rehydration obtained is shown in Table III, and can be compared with the data in Table II. The dehydrated teeth recovered some but not all of their previous moisture content. Teeth dehydrated at 100° C. and normal atmospheric pressure did not regain between 3.4 and 3.6 per cent of their original moisture. Teeth dehydrated at 197° C. in vacuo did not regain between 5.1 and 5.3 per cent of their original moisture.

TABLE III

PERCENTAGE REHYDRATION OF DEHYDRATED TEETH*

HOURS OF EXPOSURE	DEHYDRATED AT 100° C. NORMAL ATMOSPHERE			DEHYDRATED AT 197° C. IN VACUO		
	INCISORS AND MOLARS	INCISORS	MOLARS	INCISORS AND MOLARS	INCISORS	MOLARS
24	1.4	1.6	1.0	—	—	—
168	2.4	2.6	2.1	—	—	—
384	2.7	2.8	2.4	—	—	—
672	—	—	—	2.8	3.0	2.5

*Conditioned and rehydrated at 82° F. ± 3° F. and 36 to 49 per cent relative humidity; 10 teeth were used in each determination.
— = No data available.

DEHYDRATION AND REHYDRATION OF TEETH AND TOOTH TISSUES CONDITIONED AT 98° F. AND 100 PER CENT RELATIVE HUMIDITY

Whole teeth and powdered enamel and dentin (particle size of each powdered tissues, +60 −100 mesh) were conditioned to a constant weight at 98° F. and 100 per cent relative humidity. After conditioning, teeth and tissues were dehydrated in turn at 61° C. in vacuo, at 100° C. in vacuo, and at 197° C. in vacuo (Table IV). The moisture content of the teeth conditioned at 98° F. and 100 per cent relative humidity was quite similar to the moisture content of the teeth conditioned at 82° F. ± 3° F. and 36 to 49 per cent relative humidity. However, powdered enamel and dentin, conditioned at 98° F. and 100 per cent relative humidity, contained considerably more moisture than did similar tissues conditioned at 82° F. and 36 to 49 per cent relative humidity. After dehydration, both teeth and tooth tissues were rehydrated at 98° C. and 100 per cent relative humidity. It was impossible, however, to restore such

TABLE IV

PERCENTAGE MOISTURE CONTENT OF TEETH AND TOOTH TISSUES EQUILIBRATED AT 98° F. AND 100 PER CENT RELATIVE HUMIDITY*

MATERIAL	NUMBER OF TEETH OR TISSUE SAMPLES	DEHYDRATED AT 61° C. IN VACUO	DEHYDRATED AT 100° C. IN VACUO	DEHYDRATED AT 197° C. IN VACUO
Teeth, all types	10	6.5 ± .23	7.4 ± .22	8.2 ± .27
Incisors and cuspids	5	7.1 ± .20	8.0 ± .23	9.1 ± .20
Molars	5	6.0 ± .19	6.9 ± .16	7.9 ± .20
Enamel (+60 -100 mesh)	5	1.7 ± .07	2.1 ± .05	2.2 ± .04
Dentin, incisor, and molar (+60 -100 mesh)	5	12.3 ± .08	13.2 ± .13	14.5 ± .13
Dentin, molar (+60 -100 mesh)	5	12.3 ± .16	14.5 ± .13	15.6 ± .15

*Values expressed as mean and standard error.

teeth and tooth tissues to their former state of hydration (Table V). Whole teeth dehydrated at higher temperatures rehydrated less than teeth dehydrated at lower temperatures. Rehydration of powdered enamel and dentin was not affected similarly by dehydration at variable temperatures.

DISCUSSION

Considerable variation has been reported in the moisture content of the calcified tissues of human teeth. Le Fevre, Bale, and Hodge[5] reported that the inorganic portions of the calcified tissues of fetal teeth contained 6.81 per cent moisture. Le Fevre and Manly[6] reported that the moisture content of enamel averaged 2.3 per cent, with a maximum of 5.0 per cent and a minimum of 1.0 per cent. Dentin was found to have an average moisture content of 13.2 per cent, with a maximum of 15.7 per cent and a minimum of 10.8 per cent. Bird, French, Woodside, Morrison, and Hodge[2] found deciduous enamel to contain 2.8 per cent moisture, while deciduous dentin contained 11.1 per cent moisture. They compared the values for deciduous tooth tissues to those of enamel and dentin of permanent teeth which were found to contain 2.3 per cent and 13.2 per cent moisture, respectively.

In the present study, freshly extracted whole teeth without pulps were found to contain approximately 9.3 per cent moisture when determinations were made by the most rigorous procedure which presumably caused little or no degeneration of the organic matrix. Under similar conditions the moisture content of dentin from freshly extracted teeth was established at slightly more than 12 per cent of the tissue. A satisfactory procedure was not found for determining the moisture content of enamel from freshly extracted teeth. It may be that tissues from such teeth contain additional moisture but, if they were subjected to more rigorous procedures than those used in the present experiments, degeneration products could confuse the determination of water content. Obviously, procedures which result in degeneration of the tooth tissue would not be useful in establishing an equable tissue for analytic purposes.

TABLE V
PERCENTAGE MOISTURE CONTENT OF TEETH AND TOOTH TISSUES LOST AND REGAINED DURING DEHYDRATION AND REHYDRATION*

MATERIAL	NUMBER OF TEETH OR TISSUE SAMPLES	DEHYDRATED AT 61° C. IN VACUO		DEHYDRATED AT 100° C. IN VACUO		DEHYDRATED AT 197° C. IN VACUO	
		% REHYDRATION	% NOT REGAINED	% REHYDRATION	% NOT REGAINED	% REHYDRATION	% NOT REGAINED
Teeth, all types	10	5.99 ± 0.19	0.53 ± 0.02	6.46 ± 0.18	0.87 ± 0.01	6.84 ± 0.25	1.5 ± 0.10
Incisors and cuspids	5	6.42 ± 0.20	0.68 ± 0.10	6.80 ± 0.28	1.0 ± 0.05	7.35 ± 0.15	1.8 ± 0.07
Molars	5	5.56 ± 0.17	0.38 ± 0.04	6.12 ± 0.15	0.80 ± 0.05	6.33 ± 0.03	1.3 ± 0.15
Enamel†	5	1.00 ± 0.03	0.66 ± 0.06	1.14 ± 0.27	1.1 ± 0.18	1.06 ± 0.05	1.1 ± 0.06
Dentin, incisor, and molar†	5	10.94 ± 0.32	1.4 ± 0.34	11.40 ± 0.27	1.8 ± 0.35	—	—
Dentin, molar†	5	10.74 ± 0.10	1.4 ± 0.11	10.80 ± 0.29	3.7 ± 0.20	—	—

*Teeth conditioned at 98° F. and 100 per cent relative humidity before and after dehydrating at the temperatures indicated; values expressed as mean and standard error.

†All separated tissues +60 −100 mesh particle size.

— = No data available.

The moisture content of teeth eventually equilibrates with that of the environment in which they are placed once they are removed from the oral cavity. The moisture content of whole teeth previously equilibrated at approximate summer room temperature and humidity of the laboratory (82° F. and 36 to 49 per cent relative humidity) was established by the most rigorous methods to be about 8 per cent. Thus, calcified tooth tissues equilibrated at room temperature contained approximately 1.3 per cent less moisture than did tissues from freshly extracted teeth. The moisture content of powdered dentin maintained at 82° F. and 36 to 49 per cent humidity was slightly more than that of whole teeth under similar circumstances. Powdered dentin of smaller particle size contained more moisture than did dentin of larger particle size. The particle size of powdered enamel also had a similar effect on its moisture content. When the particle size of enamel equilibrated at room temperature was less than 100 mesh, it contained about 0.8 per cent moisture; when particle sizes ranged from 60 to 100 mesh, the moisture content was less than 0.4 per cent. The moisture values obtained for enamel equilibrated at room temperature were much less than those previously reported.[2, 6]

Dehydration of whole teeth and powdered enamel and dentin at a temperature of 197° C. removed more moisture than did dehydration at 100° C. Because of the probable effect of the higher temperature on the organic components of calcified tooth tissues, the most practical approach to obtaining rapidly an equable tissue with regard to moisture content is to dehydrate to a constant weight in vacuo at 100° C. It was found that the time required for dehydration at 100° C. in vacuo was 24 hours for whole teeth and powdered enamel, and 48 hours for powdered dentin.

In attempting the rehydration of dehydrated teeth and tooth tissues, it was not possible to completely rehydrate such teeth or tissues. Apparently, once the moisture has been lost from calcified tooth tissues it is not completely recovered, even in a saturated atmosphere at body temperature. In a saturated atmosphere at body temperature, whole teeth contain only a smaller amount of moisture than if they were conditioned at room temperature. On the other hand, powdered enamel and dentin contain considerably more moisture when conditioned in a saturated atmosphere at body temperature when compared to tissues conditioned at 82° ± 3° F. and 29 to 43 per cent relative humidity.

Investigations have been made of the moisture content of bone, and comparisons between the water content of bone and calcified tooth tissues are interesting. The water content of bone appears to be variable, depending in part upon the source and age of the bone and in part on the method of determination. As early as 1894, Gabriel[10] reported the water of crystallization of bone (humerus) was 2.46 per cent, while structural water was reported to be 1.32 per cent. Water of crystallization was considered the moisture released from bone at 300° to 350° C., while structural water was that which escaped at red heat. Steindler[11] found the thigh bone of an adult to be composed of 37.5 per cent water and organic material while the same bone in a newborn baby contained 64.6 per cent organic material and water. Job and

Swanson[12] dried fetal bone at 105° C. and found that it contained an average of 44.8 per cent extracellular water and 8.3 per cent intracellular water. Vogt and Tønsager[13] found considerable variation in the moisture content of bone, reporting it to range from 30 per cent at birth to 20 per cent in old age. Later, Hutchinson[14] reported the water content of bone to be 27.6 per cent. Edelman, James, Baden, and Moore[15] found that the water content of human cortical bone varied from 13 per cent to 22 per cent, while the water content of cancellous bone varied from 32 per cent to 52 per cent. Eastoe and Eastoe[16] powdered bone and allowed it to equilibrate in air for 48 hours. When heated for 24 hours at 105° C., the water content of such bone was about 8 per cent. Robinson and Elliott[17] determined the moisture content of bone by several procedures and concluded that oven drying at 100° to 105° C. was a suitable method. The water content of the cortical bone was found to be 73 per cent by volume (54 per cent by weight) in young dogs, and 21 per cent by volume (10 per cent by weight) in older dogs. In would seem, therefore, that the water content of bone varies with the source and decreases with age. Both human enamel and dentin contain much less water than bone. The water content of enamel or dentin apparently does not decrease with age as it does in bone.

SUMMARY

The moisture content of freshly extracted whole teeth and dentin was determined at 100° C. in vacuo and at 197° C. in vacuo. The maximum moisture content of freshly extracted whole teeth and dentin was established, respectively, at 9.32 ± .37 per cent and 10.00 ± .55 per cent. The maximum moisture content of whole teeth and powdered enamel and dentin previously conditioned at 82° ± 3° F. and 36 to 49 per cent relative humidity was established respectively, at 8.0 ± .24 per cent, 0.81 ± .03 per cent, and 8.7 ± .12 per cent when subjected to 197° C. in vacuo. Rehydration of such tissues at 82° F. and 36 to 49 per cent relative humidity restored some but not all the moisture. Teeth, enamel, and dentin conditioned at 98° F. and 100 per cent relative humidity contained 8.2 ± .27 per cent, 2.2 ± .04 per cent, and 15.6 ± .15 per cent moisture, respectively. Rehydration of such tissues at 98° F. and 100 per cent relative humidity restored some but not all of their moisture content.

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STUDIES OF THE COMPOSITION OF TEETH

VIII. The Composition of Human Teeth

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ALTHOUGH investigations of the composition of human teeth were begun in the latter part of the eighteenth century, valid data were not obtained until at least 1935 or later. The invalidity of the early data with regard to specific tissue composition resulted mainly from failure to separate enamel and dentin, prior to analysis. Consequently the results obtained were largely an unbalanced average between enamel and dentin, with the balance favoring dentin because it composes the bulk of the tooth. Reviews and summaries of most of the analyses of teeth or calcified tooth tissues prior to 1949 were made by Wainwright,[1] Crowell, Hodge, and Line,[2] and Leicester.[3] Subsequent to 1949, several studies and reviews have been made of the principal inorganic constituents of human enamel and dentin,[4-10] and some attention was given to the carbonate content of such tissues.[11-15] Some of the inorganic elements present in relatively small quantities of human enamel and dentin which have been investigated are iron,[16, 17] tin,[18, 19] lead,[18, 20, 21] and copper.[20, 22] Fluorides, while a minor constituent of tooth tissues, have been determined in enamel and dentin.[23-29] The organic components of enamel and dentin which have been investigated include amino acids,[30-41] lipids,[42] citric acid,[43, 44] chondroitin sulfuric acid and carbohydrates,[45-50] and total protein and organic content of tooth tissues.[51-74] Previous investigations on the composition of the calcified tissues of Syrian hamsters have indicated that the method of preparation of the tissues prior to analysis can have a significant influence on their composition.[75-78] In view of the importance of establishing as precisely as possible the composition of human calcified tooth tissues, a study, which is the basis of the following report, was made of the organic and inorganic composition of human enamel and dentin, utilizing the technics developed in previous studies of the composition of Syrian hamster's teeth.[75-78]

EXPERIMENTAL PROCEDURES

Enamel and dentin used for analysis were obtained from randomly selected caries-free human teeth divided into 3 groups as incisors, canines, and molars. Incisor enamel and dentin were obtained from an equal combination of a total of 96 upper and lower first and second incisors. Canine enamel and dentin were obtained from equal combinations of a total of 48 upper and lower canines. Molar enamel and dentin were obtained from an equal combination of a total

Received for publication July 30, 1957; revised by authors April 19, 1958.

of 96 upper and lower premolars and upper first and second molars. The enamel and dentin from each group of teeth were separated and purified as described previously.[39-41, 75-78] The moisture content of such tissues was standardized according to the procedures previously recommended by Burnett and Zenewitz.[79] The purified tissues from each group were divided into equal parts for subsequent analytical procedures. Before removing the organic portion of the samples of enamel and dentin, their nitrogen content was determined by standard micro-Kjeldahl analysis.[80] The organic content of the 2 samples of each group of tissues was determined, respectively, by extraction with 5 per cent KOH in reagent grade ethylene glycol,[78] and by heating to a constant weight at 900° C. In the case of KOH-ethylene glycol extracted samples, the inorganic residues were washed with demineralized water until the washings were neutral to litmus. All washings were added to the extraction supernatants for subsequent analysis for calcium, phosphorus, and magnesium. After extraction of the organic fraction of the samples, all residues were dried to a constant weight[79] and their nitrogen content was again determined by micro-Kjeldahl analysis.[80] The dried enamel and dentin residues were divided into suitable samples and dissolved in 0.1 N HCl for analysis. Calcium was determined by the method of Sobel and Sklersky,[81] and Sobel and Sobel,[82] phosphorus was determined by the method of Fiske and Subbarow,[83] with modification by Sobel,[84] while magnesium was determined by the method of Orange and Rhein.[85]

INORGANIC AND ORGANIC CONTENT OF HUMAN ENAMEL AND DENTIN

The organic content of human enamel and dentin is shown in Table I and compared with the inorganic residues remaining after various types of extraction. Both the nitrogen and protein content of enamel and dentin were fairly constant regardless of whether the tissue was obtained from incisor, canine, or molar teeth. The mean nitrogen and protein contents of enamel were found to be 0.065 ± .003 per cent and 0.40 ± .004 per cent, respectively. For dentin, the average nitrogen and protein contents were established at 3.43 ± .04 per cent and 21.4 ± .23 per cent, respectively.

The organic component removed by extracting the calcified tissues with KOH-ethylene glycol was greater in every instance than was the amount of protein calculated on the basis of the nitrogen analysis. In enamel, an average of 30.0 per cent more organic material was removed by extraction, than protein was found based on nitrogen analysis; in the case of dentin, however, the increase of organic material over calculated protein averaged only 3.7 per cent.

Ashing enamel at 900° C. removed much more of the tissue than was removed by KOH-ethylene glycol extraction. Likewise, the calculated protein content of enamel, as established by nitrogen analysis, was less than was removed by ashing such tissues. The amount of material removed by ashing the tissue at 900° C. averaged 6.58 per cent more than was removed by KOH-ethylene glycol extraction and 6.70 per cent more than was found as calculated protein

established by nitrogen analysis. With dentin, 5.8 per cent more of the tissue was removed by ashing at 900° C. than was removed by extracting with KOH-ethylene glycol. Likewise 6.6 per cent more of the dentin was removed at 900° C. than was found as calculated protein based on nitrogen analysis. The inorganic residue remaining after extraction of the tissue with KOH-ethylene glycol or by ashing at 900° C. was proportional to the amount of organic material determined by the particular methods employed. As was the case with the organic components of enamel, a 6.6 per cent difference was found between the amount of the inorganic residues of KOH-ethylene glycol extracted enamel and ashed enamel. A similar difference (5.8 per cent) was found between the inorganic residues of KOH-ethylene glycol extraction and ashed dentin.

TABLE I

THE ORGANIC AND INORGANIC CONTENT OF HUMAN ENAMEL AND DENTIN FROM DIFFERENT TEETH AS DETERMINED BY VARIOUS METHODS

		ORGANIC OR OTHER LABILE COMPONENTS (PER CENT)			INORGANIC RESIDUE (PER CENT)	
TISSUE*	NITROGEN CONTENT (%)	PROTEIN (N × 6.25)	KOH-ETHYLENE GLYCOL EXTRACTION	ASHING (900° C.)	KOH-ETHYLENE GLYCOL EXTRACTION	ASHING (900° C.)
Incisor enamel	0.0650	0.40	0.52	8.30	99.48	91.70
Canine enamel	0.0661	0.41	0.53	6.55	99.47	93.45
Molar enamel	0.0642	0.40	0.52	6.40	99.48	93.60
Incisor dentin	3.36	21.0	22.2	27.8	77.75	72.25
Canine dentin	3.44	21.5	22.4	28.2	77.60	71.80
Molar dentin	3.48	21.8	21.8	28.0	78.15	72.00
All types† of enamel	0.065 ± .003	0.40 ± .004	0.52 ± .004	7.1 ± .45	99.5 ± .004	92.9 ± .60
All types† of dentin	3.43 ± .04	21.4 ± .23	22.2 ± .14	28.0 ± .14	77.8 ± .15	72.0 ± .14

*Three analyses made of each of two samples of tissue from each source.
†Mean and standard error.

NITROGEN CONTENT OF THE EXTRACTED RESIDUES OF ENAMEL AND DENTIN

Since there was less material removed from enamel and dentin by KOH-ethylene glycol extraction than by ashing, the nitrogen content of the residues of KOH-ethylene glycol extracted tissues was determined. In the residues of molar enamel and incisor and canine dentin, no nitrogen was detectable by the method used for analysis. Incisor and canine enamel and molar dentin contained 0.010 per cent, 0.004 per cent, and 0.0055 per cent nitrogen, respectively.

INORGANIC CONTENT OF ENAMEL AND DENTIN EXTRACTION FLUIDS

In order to check further into the difference between the values obtained by KOH-ethylene glycol extraction and ashing, the KOH-ethylene glycol extraction fluids were analyzed for calcium, phosphorus, and magnesium and the results presented in Table II. There was a small amount of calcium, phosphorus, and magnesium in all of the extraction fluids of enamel and dentin from the various sources. The amounts of calcium, phosphorus, and magnesium

detected were not proportional to the concentration of the particular ion found in the tissue for, in most instances, quite similar amounts of these inorganic ions were found in an extraction fluid of tissue from different sources.

TABLE II
INORGANIC CONTENT OF KOH-ETHYLENE GLYCOL EXTRACTION FLUIDS OF HUMAN TOOTH TISSUES

	CALCIUM (PER CENT)		PHOSPHORUS (PER CENT)		MAGNESIUM (PER CENT)	
TISSUE*	WHOLE TISSUE	INORGANIC COMPONENT OF TISSUE	WHOLE TISSUE	INORGANIC COMPONENT OF TISSUE	WHOLE TISSUE	INORGANIC COMPONENT OF TISSUE
Incisor enamel	0.014	0.014	0.034	0.034	0.013	0.014
Canine enamel	0.014	0.014	0.040	0.041	0.014	0.014
Molar enamel	0.014	0.014	0.040	0.040	0.005	0.005
Incisor dentin	0.015	0.019	0.338	0.434	0.013	0.016
Canine dentin	0.017	0.022	0.306	0.394	0.017	0.022
Molar dentin	0.007	0.010	0.178	0.229	0.006	0.008
All types† of enamel	0.014 ± .0002	0.014 ± .0002	0.038 ± .002	0.038 ± .002	0.010 ± .003	0.010 ± .003
All types† of dentin	0.013 ± .003	0.017 ± .003	0.274 ± .044	0.352 ± .062	0.012 ± .003	0.015 ± .004

*Three analyses made of each of two samples of tissue from each source.
†Mean and standard error.

THE INORGANIC COMPOSITION OF ENAMEL AND DENTIN

The inorganic composition of KOH-ethylene glycol extracted and ashed human enamel and dentin calculated on the basis of the wet weight of the tissue is tabulated in Table III.

In the case of the data calculated on the basis of the weight of dried, whole tissues extracted with KOH-ethylene glycol (Table III), the mean percentage calcium of enamel from incisors, canines, and molars was 34.24 ± 0.42, while the mean percentage of phosphorus was 17.57 ± 0.34. The Ca:P ratio of enamel ranged from 1.93 ± 0.04 to 1.97 ± 0.05, with a mean of 1.95 ± 0.01. The mean percentage magnesium of enamel was 0.24 ± 0.02. The inorganic content of dentin extracted with KOH-ethylene glycol differed in composition from that of enamel extracted in a similar manner, particularly in regard to magnesium content. The mean percentage magnesium content of dentin was 0.94 ± 0.03, greatly exceeding that of enamel. The mean percentage calcium and phosphorus found in dentin were less than those found in enamel, being, respectively, 24.57 ± 0.07 and 12.41 ± 0.14. The Ca:P ratio of dentin ranged from 1.94 ± 0.05 to 2.02 ± 0.04, with a mean of 1.98 ± 0.02.

In the case of the data calculated on the basis of the weight of dried, whole enamel and dentin ashed at 900° C. (Table III), there was a general tendency to find approximately the same proportional amount of calcium and phosphorus as was found in the KOH-ethylene glycol extracted tissues. The mean percentage calcium and phosphorus content of ashed enamels from incisors, canines, and molars was 34.96 ± 0.59 and 16.78 ± 0.08, respectively. The Ca:P ratio of the ashed enamel was slightly more than that of the extracted tissue, ranging from 1.99 ± 0.03 to 2.12 ± 0.01, with a mean of 2.08 ± 0.04 in comparison to

TABLE III

THE PERCENTAGE INORGANIC COMPOSITION OF HUMAN ENAMEL AND DENTIN*

SOURCE OF TISSUE	KOH-ETHYLENE GLYCOL† EXTRACTED TISSUES				TISSUES ASHED AT 900° C.‡			
	Ca	P	Mg	Ca:P RATIO	Ca	P	Mg	Ca:P RATIO
Incisor enamel	33.33 ± .03	16.90 ± .43	0.22 ± .01	1.97 ± .05	33.79 ± .29	16.94 ± .41	0.20 ± .01	1.99 ± .03
Canine enamel	34.67 ± .90	17.98 ± .04	0.28 ± .01	1.93 ± .04	35.56 ± .21	16.66 ± .36	0.23 ± .01	2.13 ± .04
Molar enamel	34.72 ± .06	17.84 ± .21	0.23 ± .01	1.95 ± .02	35.54 ± .34	16.74 ± .09	0.21 ± .01	2.12 ± .01
Incisor dentin	24.51 ± .75	12.64 ± .06	1.00 ± .03	1.94 ± .05	27.12 ± .38	12.48 ± .21	0.90 ± .01	2.17 ± .03
Canine dentin	24.48 ± .55	12.14 ± .02	0.93 ± .02	2.02 ± .04	26.54 ± .12	11.57 ± .23	0.87 ± .02	2.29 ± .04
Molar dentin	24.71 ± .48	12.44 ± .07	0.88 ± .01	1.98 ± .02	26.92 ± .26	12.51 ± .30	0.84 ± .01	2.15 ± .03
All types† of enamel	34.24 ± .42	17.57 ± .34	0.24 ± .02	1.95 ± .01	34.96 ± .59	16.78 ± .08	0.21 ± .01	2.08 ± .04
All types† of dentin	24.57 ± .07	12.41 ± .14	0.94 ± .03	1.98 ± .02	26.86 ± .27	12.19 ± .32	0.87 ± .02	2.20 ± .04

*Calculated on the basis of the weight of dried, whole tissue. Values expressed as mean and its standard error.
†Three analyses made of each of two samples of tissue from each source.
‡Four analyses made of each of two samples of tissue from each source.

mean of 1.95 ± 0.01 for the extracted tissue. The mean percentage magnesium content of the ashed and extracted enamel was 0.21 ± 0.01 and 0.24 ± 0.02, respectively.

The calcium and phosphorus content of ashed dentin was somewhat similar to that of extracted dentin. The mean percentage calcium content of the ashed tissue was 26.86 ± 0.27 as compared to 24.57 ± 0.07 for extracted tissue. The mean percentage phosphorus content of ashed dentin was 12.19 ± 0.82 as compared to 12.41 ± 0.14 for extracted dentin. The Ca:P ratio of ashed dentin ranged from 2.15 ± 0.03 to 2.29 ± 0.04, with a mean of 2.20 ± 0.04 as compared to a mean Ca:P ratio of 1.98 ± 0.02 for extracted dentin.

DISCUSSION

In determining the composition of the calcified tissues of human teeth, the results obtained must be considered carefully in relation to the procedures used to prepare and perhaps analyze the tissues.

Separation and purification of enamel and dentin have been fairly well developed and established as (a) grinding of whole teeth minus pulps, and separation of the powdered tissues by flotation procedures, (b) grinding away and collection of the individual calcified tissues of the tooth, or (c) mechanical separation of tissues by chipping or breaking of teeth. All such procedures have certain defects. Procedure (a), used in the present investigation, has been used most often but it subjects tooth tissues to the action of organic solvents prior to analysis. This action could affect any tissue component soluble in such solvents. Gilda has also suggested that the flotation method produces varying samples of tooth tissue.[86] The method employed in the present investigation for separating and purifying enamel and dentin presumably removed any fat soluble material before analytical procedures were begun, for fat solvents were used prior to and during separation and purification of the tissues.[79] Furthermore, certain portions of the tissues of varying densities may be eliminated during the separation procedures. If procedure (b) had been used the tissues could have become contaminated from the grinding mechanism. It is also difficult to obtain sufficient tissues for analytical purposes, and representative samples of tissue are not always obtained. In addition to possibly affecting the organic components of tooth tissue, procedures (a) and (b) may also influence the determination of the inorganic portions of tooth tissues. As an example, Armstrong and Brekhus[87] found the carbonate content of normal enamel prepared for analysis by the flotation procedure [procedure (a)] to be 3.0 per cent, while Bowes and Murray[88] and Coolidge and Jacobs[13] found that the carbonate of normal enamel prepared by grinding [procedure (b)] was 1.95 per cent and 1.92 per cent, respectively.

Procedure (c) has seldom been used after the development of the flotation method of tissue separation because of the difficulty of obtaining sufficient amounts of tissue for analysis, because representative samples of tissue are not always obtained, and because it is difficult to obtain uncontaminated tissues. While procedure (a)—the flotation method—was used in the current investigation, it is not entirely a satisfactory method for obtaining tissue for analysis.

The moisture content is a variable factor which must be dealt with in obtaining suitable tissues for analysis, and this has been the subject of review and study.[79] The use of enamel or dentin with varying moisture content could influence particularly the determination of protein or organic components of calcified tissues when their initial weight is used as the basis of calculation. Varying moisture contents would also affect the relative concentration of the inorganic components of calcified tissues. Any moisture in calcified tooth tissues is also likely to be confused with other heat-labile substances when relatively high temperatures are used to eliminate the organic components. While the procedures used for establishing equable tissues with regard to moisture in the present investigation are the best which we could devise they are not entirely satisfactory because they allow some moisture to remain in the tissue.

Even if suitable calcified tissues are obtained for analysis, the procedures for determination of organic content and production of an inorganic residue for analysis have given results which are subject to varying interpretations. Lipids, citric acid, carbohydrates, chondroitin sulfuric acid, proteins, and other organic components have been demonstrated in enamel and dentin. Hess, Lee, and Peckham established the total lipid content (less cholesterol) of enamel and dentin to be 0.60 per cent and 0.36 per cent, respectively.[42] However, the methods used in the present investigation would eliminate most lipids before the analytical procedures were begun; hence they are not a part of the total organic content of the tissues.

The citric acid content of dentin has been reported to range between 0.80 per cent and 0.89 per cent while that of sound enamel is much less, being near 0.10 per cent.[44, 45] Since the citric acid in calcified tooth tissues is not affected by flotation procedures it would therefore be present in the tissues when analysis was begun in the present investigation.[43] Since the citric acid of tooth tissues is present as an insoluble complex containing calcium and phosphorus, it would probably be accounted for only when the organic content of the tissues was determined by heating at 900° C.

The chondroitin sulfuric acid content of dentin was found to be 0.64 per cent according to Hess and Lee.[47] The glucose content of dentin was found by Volker to range from 0.042 per cent to 0.128 per cent.[48] Stack reported the carbohydrate content of enamel to be 0.015 per cent[49] while Egyedi and Stack found a value of 0.025 per cent.[50]

According to the results reported above, the lipid, citric acid, and carbohydrate content would account for a maximum of 1.515 per cent of enamel while the lipid, citric acid, chondroitin sulfuric acid, and carbohydrate would account for a maximum of 1.228 per cent of dentin.

In addition, the protein and total organic content of enamel and dentin have been investigated. Robinson reviewed the organic content of enamel in 1945 and concluded that it varied from 0.15 per cent to 1.95 per cent based on nitrogen analysis and approximately 5 per cent based on the incineration method of analysis.[51] Losee and Hess reported the possible protein content of

enamel based on nitrogen content to be 0.46 per cent while the possible total organic content was 0.2 per cent to 1.1 per cent.[53] Stack, in 1951, reviewed the literature on the organic constituents of dentin and found that the majority reported from 25 to 28 per cent organic matter as determined by incineration.[55] His own investigations indicated that dentin contained an average of 3.44 per cent nitrogen (21.5 per cent protein) and 28.0 per cent organic matter based on incineration. Carter, Leopold, and Hess reported that dentin contained an average of 3.26 per cent nitrogen (20.3 per cent protein) and an average of 28.92 per cent organic matter as determined by incineration.[59]

In the present investigation, care was exercised during analytical procedures to account for both the organic and inorganic components of the tissues. All such analytical procedures employed to determine material not inorganic in nature, however, consistently detected variable amounts of such components in the tissue. Enamel was found to contain an average of 0.4 per cent protein, and dentin contained 21.4 per cent. Enamel contained 7.1 per cent heat-labile material while dentin contained 28 per cent. These findings are consistent with the findings of Stack[55] and Carter, Leopold, and Hess.[59] Our own findings indicate only a slight difference in the protein and KOH-ethylene glycol extractable material in either enamel or dentin. Ashing removed on an average of 6.58 per cent additional material from enamel and 6.8 per cent from dentin. The material removed by ashing at 900° C. is certainly moisture in part and other investigators have considered the other heat-labile component to be CO_2. Thus, Losee and Hess reported that enamel lost 4.81 per cent when heated to ash at a constant weight.[53] Pincus considered the CO_2 lost during ignition to be 3 per cent and that the water lost was 5 per cent.[52] However, a more positive identification of the heat-labile components of enamel and dentin is needed.

In examining the data obtained for the inorganic composition of enamel and dentin, several differences may be noted. Relatively more calcium was found in the ashed enamel and dentin than was found in the KOH-ethylene glycol extracted tissues. The amount of phosphorus tended to be more consistent in the various types of tissues regardless of how they were prepared for analysis. Considerably more magnesium was found in dentin than was found in enamel, although the amount present in either enamel or dentin was rather consistent regardless of whether the tissues were ashed or extracted.

SUMMARY

Separated and purified enamel and dentin from incisors, canines, and molars were analyzed for nitrogen content, KOH-ethylene glycol extractable fractions, and for components labile to heat at 900° C. The tissue residues from the KOH-ethylene glycol extracted tissues and from the ashed tissues were analyzed for calcium, phosphorus, and magnesium.

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URINARY FLUORINE LEVELS OF CHILDREN AND ADULTS

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WITH the increasing prevalence of artificial fluoridation of water supplies, fluorine metabolism studies have gained much in importance. Previous studies have demonstrated that the fluorine level in urine parallels the level of fluorine in the drinking water.[1-5] McClure and Kinser,[6] Likins, McClure and Steere,[7] and Zipkin, Likins, McClure, and Steere[8] investigated the fluorine content of the urine of adults drinking fluorine free or naturally fluoridated water,[6] and of adults and schoolchildren drinking defluoridated[7] or artificially fluoridated water.[8] They found that the fluorine content of the urine of adults corresponded closely to the natural fluorine content of the domestic waters they were drinking. In places where the domestic waters were essentially free of fluorine, the fluorine content of the urine of adults contained 0.3 to 0.5 ppm fluorine. On artificially fluoridating such water to 1 ppm, the fluorine concentration of the urine of adults drinking it rose within 1 to 6 weeks to 1 ppm, but the fluorine concentration of the urine of 5- to 14-year-old school children remained low (about 0.5 ppm) and rose to 1 ppm 3 to 5 years later, only. The data of their studies thus support the suggestion that the growing organism retains somewhat more fluorine than the mature organism.

From this point of view it was interesting to study the fluorine excretion in urine of younger children[9] and the following paper deals with an investigation which compares fluorine in urine of young children with that in urine of adults, both groups drinking from the same water supply.

MATERIAL

Urine specimens were collected from 59 children (1-6 years old), and 34 adults (20-55 years old), all residing in Jerusalem. This city is supplied with drinking water containing 0.5 to 0.6 ppm fluorine.[10, 11] As it was practically impossible to collect 24-hour urine samples, 1 to 4 samples from each person were taken between 9 A.M. and 3 P.M. on different days. We considered this method of sampling adequate, since fluorine concentrations determined on several urine samples from the same person on different days agreed with the average urinary fluorine content within the experimental error of the analytical method used.

Received for publication Aug. 11, 1957.

TABLE I

FLUORINE CONCENTRATION IN SPOT URINE SAMPLES OF THE SAME PERSONS ON DIFFERENT DAYS

SUBJECT	AGE (YEARS)	NUMBER OF SAMPLES	FLUORINE (PPM)	AVERAGE
C. G.	1	4	0.10, 0.10, 0.15, 0.05	0.10
P. D.	2	4	0.15, 0.10, 0.20, 0.15	0.15
S. B.	2	4	0.20, 0.25, 0.20, 0.15	0.20
L. M.	3	3	0.10, 0.02, 0.08	0.06
H. H.	3	4	0.10, 0.15, 0.25, 0.30	0.20
A. J.	4	3	0.45, 0.70, 0.70	0.62
R. A.	4	2	0.10, 0.05	0.07
A. L.	4	2	0.40, 0.35	0.37
R. R.	5	3	0.20, 0.05, 0.20	0.15
S. C.	5	4	0.65, 0.65, 0.80, 0.75	0.71
E. P.	6	4	0.30, 0.30, 0.15, 0.10	0.21
E. G.	6	3	0.35, 0.50, 0.60	0.55
R. J.	6	3	0.30, 0.20, 0.10	0.20
B. F.	24	3	0.55, 0.60, 0.50	0.55
I. G.	40	3	0.60, 0.45, 0.55	0.53

However, in view of possible inaccuracies arising from this sampling technic, the criterion for statistical significance demanded was stricter and instead of the usual level of two S.E., three S.E. was taken as the level of significance for the differences between means.

ANALYTICAL METHOD

Fifty cubic centimeters of urine was made slightly alkaline with NaOH. Two cubic centimeters of a 2 per cent calcium acetate solution was added as fluorine fixative. The sample was then evaporated to dryness and ashed at 400° C. The ashed sample was transferred to a Claisen flask and the chlorides precipitated with Ag_2SO_4. Fluorine was then steam distilled in the presence of H_2SO_4 at 140° to 150° C., according to Megregian and Solet,[12] in the modified distillation apparatus described in a previous publication.[13] Fluorine was determined photocolorimetrically in the distillate using the modified zirconium-alizarin reagent of Megregian and Maier.[14] The accuracy of this method is ±0.1 ppm F. The results are summarized in Table II.

The mean urinary fluorine content of the whole group of children was 0.2 ppm, which is a significantly lower mean than the mean urinary fluorine content of the adult group, which was 0.52 ppm and equal to the fluorine content of the drinking water, 0.5 to 0.6 ppm. This fact indicates a storage of fluorine during childhood and confirms, under natural conditions, the results of Zipkin, Likins, McClure, and Steere for artificially fluoridated waters.[8] In animal experiments, too, Savchuk and Armstrong,[15] Zipkin and McClure,[16] and Jackson, Tisdall, Drake, and Wightman[17] have shown that the young and growing rat stores considerably more fluorine in bones and teeth than the mature rat.

A significant difference was noted between children 1 to 3 years old and those 4 to 6 years old. The fluorine value of the younger children was only half of that for the older group, 0.14 ppm and 0.27 ppm, respectively. Thus, it seems that the younger the child the lower the fluorine concentration in the urine, and the greater the fluorine storage in the body.

TABLE II

MEAN FLUORINE CONCENTRATION IN URINES OF CHILDREN AND ADULTS: SIGNIFICANCE AND DIFFERENCES

AGE (YEARS)	NUMBER		VALUE OF FLUORIDE IN PPM			SIGNIFICANCE		
	INDIVIDUALS	SPECIMENS	MEAN*	MAX.	MIN.	AGE GROUPS COMPARED (YEARS)	AT SIGN. LEVEL OF 2 S.E.	AT SIGN. LEVEL OF 3 S.E.
1	10	13	0.12	0.20	0.05			
2	10	17	0.14	0.30	0.05	1 : 2	–	–
3	11	19	0.15	0.20	0.05	2 : 3	–	–
4	10	14	0.26	0.60	0.08	3 : 4	–	–
5	9	14	0.26	0.70	0.10	4 : 5	–	–
6	9	20	0.30	0.62	0.05	5 : 6	–	–
1-2	20	30	0.13	0.30	0.05			
3-4	21	34	0.20	0.60	0.05	1-2 : 3-4	+	–
5-6	18	34	0.28	0.70	0.05	3-4 : 5-6	–	–
1-3	31	49	0.14	0.30	0.05	1-3 : 4-6	+	+
4-6	28	48	0.27	0.70	0.05			
1-6	59	97	0.20	0.70	0.05	1-6 : 20-55	+	+
20-55	34	36	0.52	0.85	0.30			

*The mean fluorine concentration in the urines for the various age groups is based on the averages for each person.

In a few cases of 4- to 6-year-old children, the urinary fluorine content was quite as high as that of adults. The reasons for this early urine-water fluorine equilibrium are unknown to us. A further study of the tooth development in connection with the mineral metabolism of those children may help to clear up this problem.

SUMMARY

One hundred and thirty-three urine samples from 59 small children and 34 adults using the same drinking water were analyzed for fluorine.

The results indicate that young children store a considerable fraction of the ingested fluorine and that this fraction decreases with increasing age.

The author is indebted to Dr. G. Kallner from the Central Bureau of Statistics for the statistical evaluation of the results.

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THE RESPIRATION OF RAT AND RABBIT INCISOR PULP

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THE dental pulp which is composed of specialized loose connective tissue encapsulated with odontoblasts is undoubtedly the most vital and physiologically active of the tooth tissues. Although numerous histologic studies have been made of dental pulp, until recently there have been only a few physiologic and biochemical investigations because of inaccessibility and the small quantities of pulp tissues in the teeth of common laboratory animals and human beings.[1-4] As a result of these few studies, however, it has become apparent that further investigation of pulp physiology would be helpful in understanding pulp function under normal and diseased conditions, as well as serving as means of determining the in vitro effect of drugs and medicaments on pulp tissues.

One approach to the study of pulp physiology is by investigating the oxidative processes of pulp tissues by standard manometric methods. Utilizing such methods, Pincus[1] determined the respiration of human pulp tissue. He observed that the Q_{O_2} (microliters of oxygen consumed per milligram of dry weight of tissue per hour) was 0.31, that the Q_{CO_2} (microliter of carbon dioxide produced per milligram of dry weight of tissue per hour) was 0.29, and that the respiratory quotient (R.Q.) was 0.91. R.Q. is the ratio of carbon dioxide produced to the oxygen consumed by a tissue. Flieder and Fisher[2] reported that the endogenous Q_{O_2} of bovine pulp was 0.56 when oxygen was used as the gas phase in the manometer. Fisher, Schumaker, Robinson, Albers, and Sharbondy,[3] in a later study, indicated that pure oxygen as the gas phase was needed to maintain such a high level of oxygen consumption by bovine pulp tissue, for they observed that the Q_{O_2} was 0.33 when 4.6 ml. of air was used as the gas phase, 0.47 when 13.6 ml. of air was used as the gas phase, and 0.54 when 13.6 ml. of oxygen was used as the gas phase. Fisher and co-workers[4] found that zinc-eugenol paste, eugenol, calcium hydroxide, mercury, and amalgam reduced oxygen uptake of bovine pulp tissue by 18 per cent, 44 per cent, 35 per cent, 31 per cent, and 42 per cent, respectively, indicating the usefulness of manometric methods for investigating the in vitro action of drugs on pulp tissues.

All previous respiration studies of dental pulps have been made of human and bovine teeth. It seemed useful to investigate the respiration of pulps of common, readily accessible laboratory animals. The present study is the result of an investigation of the respiration of pulp tissues from the continually growing incisors of white rats and New Zealand rabbits.

Received for publication, Aug. 30, 1957; revised by authors March 24, 1958.

EXPERIMENTAL PROCEDURES

The pooled pulp tissues used for each determination of the rate of oxygen consumption or carbon dioxide production were obtained from 8 white male rats of the Sprague-Dawley strain whose weight ranged from 350 to 400 grams or from the incisor teeth of 6 male New Zealand rabbits, weighing between 6 and 8 pounds. The animals were stunned and sacrificed by decapitation. Upper and lower incisors of the animals were then quickly removed and split longitudinally. Pulps were gently stripped from pulp chambers and placed in cold (15° C.), oxygenated Krebs-Ringer solution, buffered with phosphate (pH 7.44). In stripping pulps from their chambers, the odontoblasts were removed along with the other pulp tissue and were subsequently a part of the respiring tissue (Fig. 1). For each test, pooled rat pulpal tissue with a wet weight ranging from 100 to 350 mg. or pooled rabbit pulpal tissue with a wet weight of approximately 200 mg. was placed in 3.0 ml. of oxygenated Krebs-Ringer phosphate solution (pH 7.44) in Warburg flasks of 15 ml. capacity. When only oxygen consumption of pulp tissue was determined, 3 Warburg reaction vessels were used; 2 containing only fluid functioned as thermobarometers, and 1, with both tissue and fluid. was used for determination of oxygen consumption. When both oxygen consumption and carbon dioxide production of pulp tissues were measured, 5 flasks were utilized for each determination; 2 containing only fluid functioned as thermobarometers, 1, with tissue and fluid, measured oxygen consumption, and 2, each with tissue and fluid, were used to determine carbon dioxide production. Two-tenths milliliter of 10 per cent KOH was used to absorb carbon dioxide in flasks in which oxygen determinations were being made, while 0.2 ml. of 0.5 N H_2SO_4 was used to release carbon dioxide from the reaction fluid of flasks in which carbon dioxide was being determined. In the thermobarometers, an additional 0.2 ml. of Krebs-Ringer phosphate solution was added so that all flasks contained a total of 3.2 ml. of fluid. All determinations were made at a temperature of 38° C. with oxygen as the gas phase. At the beginning of each determination, tissues were permitted to condition for 15 minutes, after which the manometers were adjusted and closed. At that time, acid in the side arm of the first carbon dioxide flask was added to the suspending fluid containing the tissue, and the amount of carbon dioxide evolved was measured after the flask had equilibrated for 15 minutes. Oxygen consumed by the tissue was determined at 15-minute intervals for 1 hour. At the end of 1 hour, acid in the side arm of the second carbon dioxide flask was added to the suspending fluid and carbon dioxide evolved was measured after the flask had equilibrated for 15 minutes. Carbon dioxide actually produced by respiring tissue during the 1-hour period was calculated as the difference in the amounts of carbon dioxide evolved in the first and second flasks. When oxygen consumption or carbon dioxide production was determined on the basis of dry weight, the tissue in each flask was carefully removed with forceps and dried to a constant weight at 56° C., usually for 18 hours. When oxygen consumption or carbon dioxide production was to be calculated on the basis of tissue nitrogen,

the entire content of each Warburg vessel was emptied into a digestion flask and nitrogen was determined by standard micro-Kjeldahl procedures.[6] In calculating gas volume, flask constants were adjusted for the volume of tissue samples, for which a density of 1.0 was utilized.

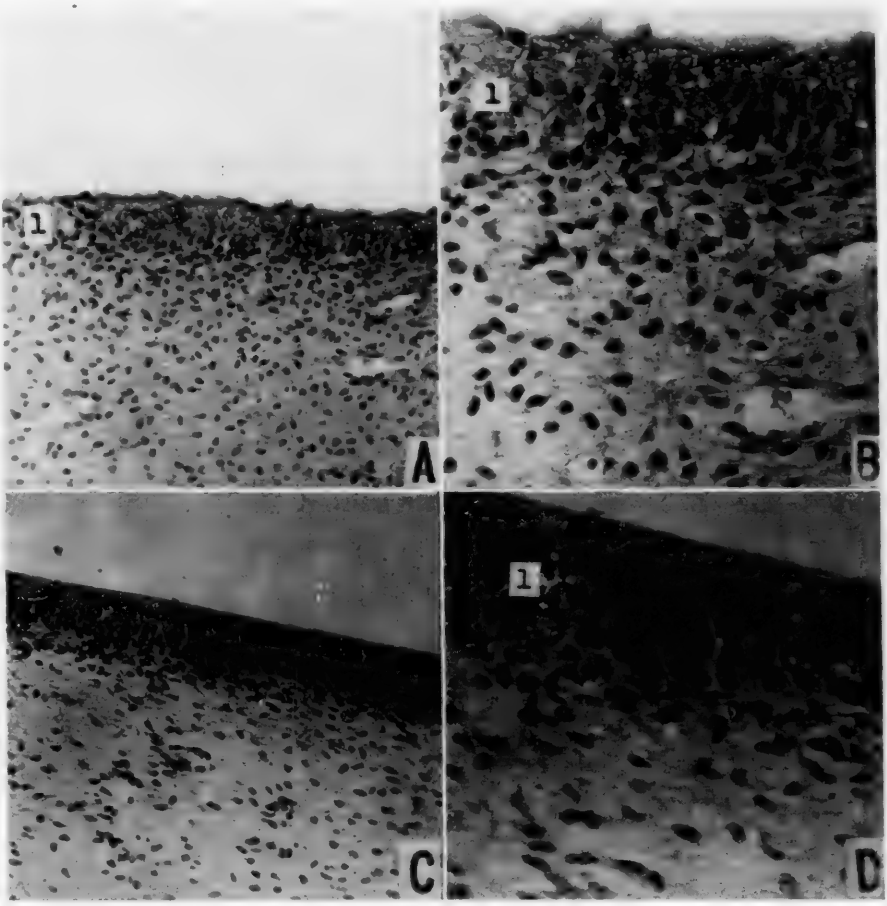

Fig. 1.—The histology of pulp tissue stripped from the pulp chamber of rat incisors (*A, B*) and rabbit incisors (*C, D*). The odontoblastic layer (*1*) is intact and is a part of the respiring tissue. (*A* and *C* × 136; *B* and *D* × 334).

RESULTS

Rat Incisor Pulps.—With incisor pulps, 3 groups of experiments were performed as noted in Tables I and III. In Group 1 (Table I), 10 determinations of oxygen consumption of pulp tissue were made and the results calculated on the basis of wet and dry weight of tissue. Mean Q_{O_2} based on wet weight was 0.2734, while mean Q_{O_2} based on dry weight was 5.10. The ratio of oxygen consumption, based on dry weight of tissue to oxygen consumption based on wet

weight of tissue, averaged 18.6:1. This compares closely to the ratio of wet to dry tissue which averaged 17.7:1. Pulp tissue contained an average of 94.7 per cent water and 5.3 per cent solid matter.

In Group 2 experiments (Table I), 10 separate determinations were made of the oxygen consumption of pulp tissue and the results are expressed on the basis of wet weight and nitrogen content. The mean Q_{O_2} based on wet weight

TABLE I
THE OXYGEN CONSUMPTION OF RAT PULP TISSUE

GROUP	NUMBER OF EXPERIMENTS	BASIS FOR ESTIMATE OF AMT. OF TISSUE	MEAN*	RANGE*	STANDARD DEVIATION	STANDARD ERROR OF MEAN	COEFFICIENT OF VARIATION
1	10	Wet weight	0.2734	0.1842- 0.3349	0.0491	0.0155	12.95
		Dry weight	5.10	4.23 - 6.84	1.01	0.319	29.80
2	10	Wet weight	0.2842	0.2408- 0.3081	0.0245	0.0077	8.62
		Nitrogen content	29.70	26.95 -35.51	2.84	0.89	9.56

*Microliters per milligram per hour.

was 0.2842. The mean Q_{O_2} was 29.70 based on the nitrogen content of the tissue. The ratio of oxygen consumption based on nitrogen content of tissue to oxygen consumption based on wet weight averaged 104.5:1.

In Group 5 experiments (Table III), 15 determinations were made of both oxygen consumption and carbon dioxide production of rat incisor pulp tissue. Oxygen consumption, carbon dioxide production, and respiratory quotients were calculated on the basis of both wet weight and nitrogen content of the tissue samples. The mean Q_{O_2} based on wet weight was 0.4054. The mean Q_{O_2} of the same tissues, calculated on the basis of nitrogen content, averaged 38.59. The ratio of oxygen consumption based on nitrogen content to oxygen consumption based on wet weight was 95.18:1. The Q_{CO_2} of rat pulp tissue based on wet weight averaged 0.3678. On the basis of the nitrogen content of the tissues, the mean Q_{CO_2} was 34.52. The respiratory quotient of pulp tissue based on either wet weight or nitrogen content was 0.90.

TABLE II
THE OXYGEN CONSUMPTION OF RABBIT PULP TISSUE

GROUP	NUMBER OF EXPERIMENTS	BASIS FOR ESTIMATE OF AMT. OF TISSUE	MEAN*	RANGE*	STANDARD DEVIATION	STANDARD ERROR OF MEAN	COEFFICIENT OF VARIATION
3	12	Wet weight	0.1479	0.1060- 0.1753	0.0223	0.0064	15.07
		Dry weight	2.48	1.54 - 2.95	0.415	0.119	16.73
4	10	Wet weight	0.1475	0.1012- 0.2170	0.0406	0.0128	27.52
		Nitrogen content	14.46	9.84 -20.04	3.48	1.10	24.06

*Microliters per milligram per hour.

Rabbit Incisor Pulps.—With rabbit incisor pulps, 3 groups of experiments were performed and the data recorded in Tables II and III. In Group 3 experiments (Table II), 12 determinations were made of the oxygen consumption

TABLE III

THE RESPIRATION OF RAT AND RABBIT PULP TISSUES

ANIMAL AND GROUP	NUMBER OF EXPERIMENTS	STATISTICAL ANALYSIS	OXYGEN* CONSUMPTION (Q_{O_2})		CARBON DIOXIDE* PRODUCTION (Q_{CO_2})		RESPIRATORY QUOTIENT (R.Q.)	
			WET WEIGHT	NITROGEN CONTENT	WET WEIGHT	NITROGEN CONTENT	WET WEIGHT	NITROGEN CONTENT
Rat	15	Mean	0.4054	38.59	0.3678	34.52	0.903	0.901
5		Range	0.2457-0.6299	27.80-61.03	0.2253-0.6000	21.49-53.26		
		Standard deviation	0.1022	9.80	0.0983	8.68	0.037	0.037
		Standard error of mean	0.0263	2.53	0.0254	2.24	0.0096	0.0096
		Coefficient of variation	25.20	25.39	26.72	25.14	4.09	4.10
Rabbit	10	Mean	0.1382	13.66	0.1348	13.22	0.972	0.967
6		Range	0.0966-0.1820	10.73-18.56	0.0958-0.1758	9.90-15.64	0.92-1.01	0.88-1.06
		Standard deviation	0.0283	2.45	0.0276	2.57	0.0301	0.0549
		Standard error of mean	0.0089	0.784	0.0087	0.815	0.0095	0.0173
		Coefficient of variation	20.47	17.93	20.47	19.44	3.09	5.67

*Micrograms per milligram per hour.

of rabbit pulp tissues and the data calculated on the basis of wet and dry weight. When wet weights were used as the basis for calculation of oxygen consumption, the tissues were blotted thoroughly before weighing, while in the case of dry weight determinations, the tissues were dried to a constant weight in an oven at 56° C., usually for 18 hours, before weighing.

In the second group of experiments (Group 4, Table II), 10 determinations were made of the oxygen consumption of rabbit pulp tissues and the data calculated on the basis of wet weight and nitrogen content of the tissues. When wet weight was used as the basis of calculation, the pulps were blotted thoroughly and weighed before placing in the fluid of the reaction vessel. When the nitrogen content of the tissue was used as the basis of calculation, pulpal tissue, together with the fluid from each Warburg flask, was transferred at the end of each experiment directly into Kjeldahl digesting vessels for determination of nitrogen.

With the Group 6 pulps (Table III), 10 separate determinations of endogenous oxygen consumption and carbon dioxide production were made and the data calculated on the basis of wet weight and nitrogen content. Wet weight and nitrogen content of tissues were determined in a manner similar to that described for Group 4 animals.

Mean Q_{O_2} based on wet weight of tissue was 0.1479, 0.1475, and 0.1382, respectively, for the incisor pulps of rabbits of Groups 3, 4, and 6. Mean Q_{O_2} based on dry weight of tissue was 2.48 for pulps of Group 3 only. Thus, Q_{O_2} based on dry weight of rabbit pulp was 16.8 times more than Q_{O_2} based on wet weight. In comparison, in the rat, Q_{O_2} based on dry weight of tissue was 18.6 times more than oxygen consumption based on wet weight of tissues. Incisor pulp tissue of rabbits was composed, on an average, of 94.04 per cent water and 5.96 per cent solid material, giving a ratio of wet to dry tissue of 15.8:1. In comparison, the ratio of wet to dry pulp tissue of the rat was 17.7:1. The mean Q_{O_2} of rabbit pulp tissue, calculated on the basis of nitrogen content, was 14.46 and 13.66, respectively, for the pulp tissues of the animals of Groups 4 and 6, with an over-all average of 14.06. The mean ratio of oxygen consumption based on nitrogen content to oxygen consumption based on dry weight was 5.7:1, and the mean ratio of oxygen consumption based on nitrogen content to the ratio of oxygen consumption based on wet weight was 98.5:1.

Carbon dioxide production was measured for rabbit pulp tissues only from the animals of Group 6. Mean Q_{CO_2} based on wet weight of tissue was 0.1348, while Q_{CO_2} based on nitrogen content was 13.22. Thus, the respiratory quotient of rabbit pulp tissues was 0.972 based on wet weight and 0.967 based on nitrogen content.

DISCUSSION

The endogenous oxygen consumption of rat incisor pulp based on dry weight of tissue and with oxygen as the gas phase ($Q_{O_2} = 5.10$) was found to be much higher than that previously reported for either human (Q_{O_2} based on dry weight $= 0.31$)[1] or bovine (Q_{O_2} based on dry weight $= 0.56$)[2] dental

pulps. Under similar conditions, the endogenous oxygen consumption of rabbit incisor pulp was from 51 to 60 per cent less than that of rat incisor pulps. The differences in the oxygen consumption of rat and rabbit pulps, and human and bovine pulps may be mediated in part by the retention or loss of the odontoblastic layer of the respiring tissue, by species differences, by such basic metabolic differences as might be related to continual growth and eruption of the teeth, or by varying experimental conditions. Differences between the endogenous oxygen consumption of rat and rabbit incisor pulps could not be attributed to varying experimental conditions or to retention or loss of the odontoblastic layer, since standardized conditions were used during each determination with these tissues. As is indicated in Fig. 1, the odontoblastic layer remained attached to both rat and rabbit pulp tissue as it was removed from the pulp chambers, making possible the determination of the respiratory activity of whole pulp. Since odontoblasts in rat and rabbit incisor teeth actively form dentin throughout the life of the animal, such continued growth and activity may be reflected in their respiratory rates.

So far as is now known, it is merely a matter of speculation whether differences in the endogenous respiration of pulp tissue are related to species differences or to basic metabolic differences.

Since experimental conditions differed in the various tests, caution must be used in comparing the respiration rates of human,[1] bovine,[2] and rat and rabbit dental pulps as in the present study. Studies on human, rat, and rabbit pulp utilized whole pulps, while tissue slices were employed in the work on bovine pulp. The work on bovine, rat, and rabbit pulps, moreover, employed an oxygen gas phase and a pH of 7.4 in the reacting medium. The pH and the gas phase are not stated in the study of human pulp. Furthermore, the criteria for "dried" tissue were not the same in the three studies. Human pulp tissue was dried for 2 hours at 100° C., bovine pulp was dried for a minimum of 6 hours at 106° C., and, in the present study, rat and rabbit dental pulps were dried at 56° C. until constant weight was obtained. It was not stated whether a constant weight of tissue was obtained in the studies on human and bovine pulps.

Wet weight, dry weight, or nitrogen content are usually used as the basis for calculating oxygen consumption and carbon dioxide production of tissues. Because of the small quantities of dental pulp that are readily available, and the error that is introduced by drying small quantities of tissue, it is believed that a more accurate basis for respiration studies is the nitrogen and hence protein content of pulp tissues. It is a more accurate and convenient method than attempting to retrieve minute quantities of tissue for dry weight procedures. Furthermore, there are many small pieces of tissue which have broken off from the main pulpal mass which are not at all retrievable in dry weight procedures although they may have contributed to the respiration. Such errors can be eliminated by analyzing for the nitrogen content of the tissue and reacting medium

by standard Kjeldahl procedures. It is the opinion of Umbreit, Burris, and Stauffer,[5] with whom we concur, that "since metabolic activity is associated more directly with the nitrogen (protein) content of a given tissue, it would seem more desirable to include nitrogen determination when comparing tissues."

Other factors which must be considered in determining the respiratory rates of tissues are the type and nature of the suspending medium, and the length of time between the death of the animal and the determination. The medium in all 3 studies has been the same, but the period of time from the accession of the pulp to the beginning of manometric procedures has varied from 30 minutes to 2 hours.

Thus, from the foregoing discussion it would seem best, with our current knowledge, not to make definitive comparisons between the respiratory rates of dental pulps from human beings and various other animal species. If, indeed, the oxygen consumption of rat dental pulp is so much greater than that of human and bovine pulp, this would not be surprising in view of the continual growth of rat incisors.

The respiratory quotient noted in our study was 0.90 for rat incisor pulp and 0.97 for rabbit incisor pulp. These values were derived from calculations based on wet weight and nitrogen content of the tissue samples. The respiratory quotient of the rat incisor pulp is similar to the 0.93 value recorded by Pincus[1] for human pulp, but the respiratory quotient of the rabbit incisor pulp is higher than that of either human or rat incisor pulp. The values obtained would seem to indicate that carbohydrate was being utilized in a similar manner by the 3 tissues even if they did vary considerably in their rate of respiration.

SUMMARY

1. The mean endogenous oxygen consumption of rat incisor pulp tissue with oxygen as the gas phase, calculated on several different bases, was found to be as follows:

Q_{O_2} (per mg. wet weight) = 0.33 μl per hr. (Groups 1, 2, 5).
Q_{O_2} (per mg. dry weight) = 5.10 μl per hr. (Group 1).
Q_{O_2} (per mg. nitrogen) = 35.04 μl per hr. (Groups 2, 5).

2. The carbon dioxide production of rat incisor pulp tissue with oxygen as the gas phase, calculated on several different bases, was found to be as follows:

Q_{CO_2} (per mg. wet weight) = 0.37 μl per hr. (Group 5).
Q_{CO_2} (per mg. nitrogen) = 34.52 μl per hr. (Group 5).

3. The respiratory quotient of rat incisor pulp tissue, calculated on several different bases, was found to be as follows:

R.Q. (wet weight) = 0.903 (Group 5).
R.Q. (nitrogen content) = 0.901 (Group 5).

4. The endogenous oxygen consumption of rabbit incisor pulp tissue, with oxygen as the gas phase and calculated on several different bases, was found to be as follows:

Q_{O_2} (per mg. wet weight) = 0.14 μl per hr. (Groups 3, 4, 6).
Q_{O_2} (per mg. dry weight) = 2.48 μl per hr. (Group 3).
Q_{O_2} (per mg. nitrogen) = 14.06 μl per hr. (Groups 4, 6).

5. The carbon dioxide production of rabbit incisor pulp tissue, with oxygen as the gas phase and calculated on several different bases, was found to be as follows:

Q_{CO_2} (per mg. wet weight) = 0.13 μl per hr. (Group 6).
Q_{CO_2} (per mg. nitrogen) = 13.22 μl per hr. (Group 6).

6. The respiratory quotient of rabbit incisor pulp tissue, calculated on several different bases, was found to be as follows:

R.Q. (wet weight) = 0.97 (Group 6).
R.Q. (nitrogen content) = 0.97 (Group 6).

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DENTAL OBSERVATIONS MADE WHILE WINTERING IN ANTARCTICA, 1956-1957

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EARLY in the initial planning of the United States National Committee for the International Geophysical Year, the Dental Division of the Bureau of Medicine and Surgery began to explore the possibilities of including a dental officer in the wintering-over party of the operation known as Deepfreeze I. The assignment of a dentist to the already formed group of 92 men was accomplished in September, 1955. Primarily, the dentist's duties were to include the emergency and routine treatment of these 92 men, but because of the unusual nature of the operation, where men would be subjected to the isolation and the physical and psychological stresses of life on a continent having a prolonged night and temperatures far below those experienced anywhere else in the world, it was hoped that some insight might be obtained on the effect of these conditions on oral health.

The course decided upon was one of clinical observation in which the dental officer would observe and report any clinical conditions that might be worthy of future investigation. Also, it was hoped that some insight might be gained in cariogenic trends determined through clinical, radiographic, and bacteriologic studies. Observations of soft tissue changes, as well as dietary desires, smoking habits, and oral hygiene procedures, were placed in the experimental outline.[1]

A landing was made at McMurdo Sound, Antarctica, the latter part of January, 1956, and, because of the activities associated with survival, it soon became apparent that definitive laboratory investigations would have to be deferred until the operation's pioneer first-year phase had passed.

REVIEW OF THE LITERATURE

References to the health and well-being of men participating in previous Antarctic expeditions are numerous. Included in these reports are frequent observations of oral health in which dental complaints play an outstanding part. According to Frazier, a physician at Little America with Admiral Byrd during the 1939-41 expedition: "Most of our problems, medically, were teeth."[2] Mountevans[3] repeatedly pointed out that in the traverse teams of almost every Antarctic expedition many men suffered from bleeding, sore, swollen gingiva and looseness of the teeth, which, in hindsight, he attributed to an improper diet lacking particularly in vitamin C.

Received for publication Aug. 31, 1957; revised by authors March 12, 1958.

Cherry-Garrard,[4] on Scott's last expedition, wrote that an almost unbelievable dental condition occurred: "In the pauses of our marching we halted in our harnesses, the ropes of which lay slack in the powdery snow. We stood panting with our backs against the mountainous mass of frozen gear which was our load. There was no wind, at any rate no more than light airs: our breath crackled as it froze. There was no unnecessary conversation: I don't know why our tongues never got frozen, but all my teeth, the nerves of which had been killed, split to pieces."

Amundsen[5] wrote of the need for adequate dental first aid equipment. Giaever[6] remarked on the deplorable state of oral hygiene noticed in his personnel, stating that "the toothbrush remains for most rather an instrument for use on ceremonial occasions." Frazier wrote[2]: "All devitalized and carious teeth became painful and had to be extracted. As soon as cold air was brought in contact with these diseased teeth, the pain became unbearable. As the cold increased with the coming of winter, the alloy fillings contracted and pulled away from the cavities they were protecting. Some fell out immediately and others began to leak resulting in decay under fillings. Many good teeth were lost in this manner. Front teeth with large fillings and gold inlays became very painful on exposure to low temperatures. The back molars, protected by the tongue and cheek, were the least affected." Frazier further stated that wintering parties have higher than usual epinephrine levels. He cited a number of cases in which varying degrees of shock were elicited through the administration of Novocain solution containing varying concentrations of Adrenalin, ranging from 8 minims to 2 minims per ounce of anesthetic solution. He finally reached a point where Adrenalin-free anesthetic solutions were used.

OBSERVATIONS ON ORAL HEALTH, 1956-57, AT MC MURDO SOUND, ANTARCTICA

Patient Load.—

Ninety-three men were stationed at McMurdo Sound, Antarctica, during the winter, 1956. Since one of these was a dentist, 92 men were eligible for dental treatment. At no time did the dentist solicit patients but 89 men voluntarily sought aid. Table I shows the month-by-month demand for dental and medical service expressed in numbers and per cent of total population. Each patient is counted but once a month regardless of the frequency of his visits or multiplicity of his complaints.

TABLE I

MONTH	NO. OF MEN UNDER DENTAL TREATMENT	PER CENT OF TOTAL POPULATION UNDER DENTAL TREATMENT	NO. OF MEN UNDER MEDICAL TREATMENT	PER CENT OF TOTAL POPULATION UNDER MEDICAL TREATMENT
April	35	38.0	40	43.5
May	39	42.4	34	37.0
June	27	29.3	30	32.6
July	32	34.8	47	51.1
August	39	42.4	54	58.7
September	47	51.5	52	56.5

The months April through September were chosen for their statistical significance for at least 3 reasons. It was during this period that the camp population remained static; there were no transient personnel. These were the months of the severe Antarctic winter, with temperatures frequently below the –50° F. mark. It was necessary for men to spend as much as 12 hours a day, 7 days a week, in preparation for the returning scientific personnel who would gather their data during the International Geophysical Year.

The number of patients seeking treatment in April and May, though relatively high, increased only slightly in May over April. In June, the men remained indoors and one would have predicted on the basis of time available for treatment, that the number of persons desiring routine treatment would have increased but there was a 13.1 per cent drop in demand. As the winter night progressed and the men returned outdoors in mid-July, the incidence of persons seeking dental aid increased each month until September when more than half of the camp personnel were under treatment.

A comparison of the demand for medical and dental treatment is interesting (Table I). Whereas the demand for dental treatment increased in May over April, the demand for medical care decreased. In June there was a greater decrease in demand for dental care than for medical care. In July, upon returning outdoors, there was a sharp increase in the need for medical services amounting to 18 per cent, increasing still higher by 7.6 per cent in August, and then apparently leveling off in September. Many of these patients sought aid for traumatic injuries while working in low temperatures during the Antarctic night.

Dental Treatment.—

The call for dental services can be broadly grouped into 3 categories: (1) teeth, (2) supporting structures, and (3) prosthetic appliances.

Much has been written by persons not trained in dental observations concerning the loss of fillings from teeth through the differences of coefficient of contraction of tooth substance and filling material when exposed to the severe cold of the South Polar continent. It seems highly improbable that this is so, since air, cold enough to cause metallic contraction of such magnitude as to bring about loss of the filling, would certainly have effects on other tissues. Armstrong and Huber[7] and others have found no measurable filling change or dislodgment when teeth were subjected to severe rapid changes in temperature. Certainly, if a vital tooth was involved, such a thermal change would cause a severe toothache. Again, if these thermal extremes were reached, we could expect some effect on the oral mucous membrane and the pulmonary tissues. Teeth were the only oral tissues reported to have been affected by the cold.

Restorations did fall out; in one day alone, there were 4 cases. In almost every instance their loss could be explained by the presence of secondary decay, occlusal trauma, or some other physical reason.

In this first expedition, time was not available before departure to check thoroughly and correct the dental deficiencies existing in the men. It is entirely

possible that, had these men had adequate treatment prior to leaving, results paralleling those of Admiral Byrd's 1928 Antarctic expedition would have been achieved. Prior to this expedition, Nelson[8] made every man "dentally fit." "Every cavity was filled with the best obtainable material and the gums given the best possible treatment. Every dental fault was corrected." Upon return of the expedition in 1930, Nelson re-examined[9] 59 of the group who had been in Antarctica for a 2-year period and reported that there were some new cavities which had developed. Of the 59 men examined, 28 had no new cavities or restorative work necessary. There were 42 new cavities in the remaining 31 men, 2 broken teeth, and 4 broken fillings. These broken restorations had been placed some time prior to 1928. These men were under professional observation and loss of restorations due to extreme temperatures was not a cold-weather dental problem. All the personnel of the Antarctic expeditions during the International Geophysical Year through 1959 will be closely observed, however, and it is hoped that a final answer to this problem will be had from the collected data.

Tooth splitting or fracture: The phenomenon of tooth splitting in cold weather, which was reported by Cherry-Garrard,[4] was not observed at any time in the personnel of Deepfreeze I. Since pre-expedition radiographs were not available, it cannot definitely be stated that any pulpless teeth of long duration existed in this group of men. Several teeth were pulp treated and the root canals filled during the expedition but these did not prove to be a problem.

In other instances, tooth cusps fractured or other forms of tooth substance were lost but, once again, professional analysis at the time attributed the splitting to secondary decay, undermined enamel, or entirely to the trauma of occlusion.

Cold and toothache: Toothache due to inhalation of cold air was a rather common complaint. In the majority of instances it occurred in teeth with metal restorations in which insulating bases were inadequate or missing. With most of these there was no previous history of odontalgia and the placement of the restorations in the involved teeth dated from 6 years to a few months prior to departure on the expedition. The histories of these patients were all quite similar. They had had no dental troubles until they went outdoors in low temperatures. The toothache was moderately severe and of durations ranging from a few hours to a few days. Many of these men attempted self-treatment by taking aspirin and, in many instances, this medication offered temporary relief from pain. The severity of the pain could not be correlated with the size of the metallic restorations because in several instances a clinically small insignificant-looking occlusal amalgam was the offender. In each case, however, these were quite deep and without insulation.

Treatment for toothaches with cold as the etiologic agent consisted of removal of the metallic restoration and replacement of it with zinc oxide and eugenol for a few days until the pulpal hyperemia disappeared. This zinc oxide and eugenol was then replaced with an amalgam and an oxyphosphate cement

base. In several instances where the cavity was particularly deep, a small amount of the sedative base was allowed to remain and the oxyphosphate base was used as an intermediate base. In every instance where pulpal deterioration had not taken place, this treatment proved successful and the tooth remained comfortable for the remainder of the stay in Antarctica.

Teeth with large unfilled cavities were not too great a problem insofar as their reaction to cold temperatures was concerned. Many patients sought routine dental treatment because of its availability at which time the dentist discovered large and sometimes very deep cavities. Upon questioning the patient about his experiences in the cold air, the answer was usually that cold air had no effect on them. From this, the only conclusion that can be drawn is that the decalcified material and food debris packed in the cavity acted as an insulating layer and that pain occurred only through the prolonged chilling of the uninsulated metallic restorations.

Another common complaint of the teeth associated with cold temperatures is pain due to the exposure of the necks of teeth. There did not seem to be any correlation between this condition and the rather common complaint of hypersensitive cervical dentin. Relief from the latter was usually realized upon the application of silver nitrate.

When restorations are placed in accordance with accepted standards, cold air as an etiological factor in odontalgia, or cold air as an etiological factor in loss of restorations, is negligible. If the carious process has progressed to any extent, an intermediate base of zinc-phosphate cement or a combination of zinc oxide-eugenol and zinc-phosphate cement should be used. The rule followed was that if there was any doubt, a cement base was used. The pleasing result was that virtually every tooth filled was comfortable from the first day and remained so for the entire stay.

Supporting Tissues.—

Alveolar osteitis ("dry socket"): During this first year in Antarctica, 44 extractions were performed. All extractions were performed under local anesthesia with instruments boiled for at least 15 minutes immediately prior to the operation and transferred from the boiling water to an autoclaved towel by means of sterile forceps. The operator's hands were scrubbed with soap and water.

Of the teeth extracted, 11 were abscessed, 16 had carious exposures, and 17 were removed electively, usually third molars having no antagonist or having had a history of being the etiological factor in a pericoronitis. Of the 44 extractions, only one could be considered difficult. Of those teeth removed in the presence of infection, antibiotics were administered at the discretion of the dentist. Immediate postoperative treatment consisted of ascertaining that an adequate blood clot had formed and giving the patient instructions about not disturbing the clot with the tongue, food, or forceful mouth rinses. The exposure of the patient to the severe cold and wind of the outdoors during the times it was necessary to walk from his quarters to other base activities for eating, working, recreation, or returning to the dental office, was unavoidable.

Alveolar osteitis followed 10 of the 44 extractions, an average of 22.73 per cent as compared with 0.9 per cent average as reported by The University of Pittsburgh School of Dentistry[10] in a study of 23,886 cases following extraction. This condition appeared both as an infected socket with a deteriorating blood clot or as a dry socket with the complete loss of the clot. The ensuing number of treatments required in a given case ranged from 1 to 19.

The etiological factor or factors were not sought during this first expedition because of the lack of facilities to carry out such an investigation. Various theories have been advanced and will be studied in future expeditions. These theories include as the etiological factor of alveolar osteitis in Antarctica: (1) viral or bacterial infection, (2) disturbances in the bleeding and clotting times as determined by the fibrinogen, thrombin-prothrombin, calcium and platelet blood levels, (3) the effect of cold itself on the clotting mechanism, (4) a general lowering of tissue resistance due to the stresses of Antarctic life, and (5) the vasoconstrictive action of epinephrine in the presence of high epinephrine blood levels.

Periodontal disorders: During this expedition there was only a small number of periodontal disorders to be treated by the dental officer. Gingival disorders usually responded to routine treatment once the etiology was ascertained. There were several cases of pericoronitis and these, too, responded favorably to treatment. In several instances of recurring pericoronitis the third molar was eventually removed.

Aphthous stomatitis appeared in about 10 per cent of the personnel. Treatment in each instance consisted of a mild astringent applied to the lesion as early as possible. No further treatment was considered necessary and complete healing was usually effected in 7 to 10 days.

Two cases of general soft tissue inflammatory condition appeared at a time after several weeks of extended work before the period of darkness arrived. The personnel had been working 12 hour shifts, 7 days a week, and there was a noticeable weariness in the men. The men had been told a few days previously that there still remained much necessary outdoor work. In one instance, a patient presented with an annoying aphthous lesion on the mucous membrane of the lower lip, and very sore and bleeding gingiva. The aphthous stomatitis was treated with a mild astringent and the patient asked to return the following morning for a scaling to treat the inflamed gingiva. Examination the next morning revealed a mouth with normally colored gingival mucous membranes. The swelling had disappeared, the soreness was gone and the gingival tissues did not bleed on pressure. The patient also stated that, physically, he felt better. A further history revealed that the patient had been under considerable tension and fatigue for several days in this prolonged outdoor work. His mouth during this time had become progressively more uncomfortable and the night before he had originally reported to the dental office he had had only 4 hours of sleep. Since appearing the day before he had a long sleep and a warm shower—the only treatment. His teeth were subsequently scaled and no further treatment was necessary.

Oral hygiene: Some previous Antarctic expeditions have reported deplorable oral hygiene practices.[6] It is presumed that this condition could exist because of the lack of basic training in the importance of a clean mouth or because of inadequate facilities to make toothbrushing possible. In all previous expeditions, camp facilities were primitive and exploratory traverses were either on foot or with dog team and lasted for weeks and even months. Toothbrushing under these conditions was almost an impossibility.

The life of our Antarctic explorers today can be compared with their predecessors in only limited areas. Traverses that took months now take hours with the airplane. Primitive camp facilities have been replaced with recreation rooms and running warm water for showers.

Mouth hygiene of Deepfreeze I personnel was generally good. The presence of excellent camp leadership was reflected in the cleanliness of the camp, and the presence of a physician and dentist was reflected in good personal and oral hygiene.

Dental calculus: Nelson examined the returning members of Admiral Byrd's expedition and reported that there was not a single case of excessive calculus deposit.[9] In observation of Deepfreeze I personnel, the deposition of calculus appears to progress at about the same rate as it would in the United States. Those persons who previously had histories of heavy deposits continued to have heavy deposits requiring frequent scalings and those persons with little tendency to calcific deposits continued to show the same trends.

Prosthetic Appliances.—

From January, 1956, to January, 1957, it was necessary to repair 8 dentures. A ninth case was seen in consultation with the dental officer attached to an ice breaker. Of these cases only 1 could be definitely attributed to the cold weather. In this instance, a man wearing a full upper denture had been standing a 4-hour watch on a cold windy day. He was a mouth breather and, during this watch, he stated he could actually feel the denture cracking as he breathed through his mouth. Subsequent examination of the denture revealed a great number of small cracks. The denture had been worn about 5 to 6 months prior to this peculiar fracturing.

Of the remaining dentures seen for repair, 5 had been broken as the result of direct trauma. The remaining 3 fractured during eating and may or may not have been the result of rapid temperature change from outdoors to the warmth of food. However, these 3 dentures were fractured in a single line and exhibited none of the "shattering" effect seen in the full denture.

Miscellaneous.—

Shock following local anesthesia: Frazier reported that "adrenalin shock"[2] frequently followed injection of a local anesthesia containing Adrenalin into camp personnel who were cold acclimatized. He theorized that this resulted from an increase in the already high epinephrine blood levels. A series of experiments were carried out by him with varying concentrations and over extended periods, and he was able to reproduce this condition at will.

On the basis of this report, the use of local anesthetics and patient reaction were carefully observed. In the use of 250 cartridges of 2 per cent lidocaine hydrochloride, containing 1:50,000 epinephrine, only 2 cases of syncope were observed. Both patients appeared at morning appointments and had had no breakfast prior to the visit to the dentist. Both patients were given the same type of anesthetic at subsequent sittings and experienced no untoward results.

Focus of infection: During the stay at the McMurdo Sound wintering camp, the study of 1 patient strongly suggested to the dental officer and medical officer that this patient's traumatic arthritis had been kept active through the influence of a dental focus of infection.

CONCLUSIONS AND SUMMARY

The presence of a dentist in an expedition to Antarctica is a necessary factor in raising the morale and tending to the dental complaints of the expedition's personnel. His importance is shown by the fact that 89 persons out of 92 sought his aid during this year's stay in Antarctica.

Odontalgia as a result of thermal conductivity through metallic restorations lacking or insufficient in intermediate cement bases is a common Antarctic dental complaint. This condition can be controlled by replacing the restoration and supplying the tooth with an adequate insulating cement base.

Hypersensitive cervical dentin was also a common complaint, controlled through the use of silver nitrate, or, if such conservative measure failed, a cavity was prepared and an insulated filling was placed. It is the firm belief of the observer that restorations placed under standard methods were easily tolerated and remained intact.

Loss of restorations through the action of the Antarctic cold did not prove to be a problem as previously reported. No marginal leakage was observed through contraction and each restoration, that did fall out, fell out because of some pre-existing condition such as secondary decay, undermined enamel, or trauma.

Observations of the soft tissues and periodontal structures indicated that, given the facilities and time, oral hygiene remained good. Generalized periodontal trouble was not a problem but 2 cases were cited where the etiology appeared to be long hours of exhaustive work at low temperatures. Several cases of aphthous stomatitis appeared and were treated with a mild astringent. These aphthae healed in the normal time of 7 to 10 days. The rate of deposition of dental calculus did not appear to be unusual or to change from what would be expected in the same individual if living elsewhere.

Alveolar osteitis was a problem and occurred once after every 4 or 5 extractions. All extractions were done under generally accepted procedures of sterility and it is the authors' belief, in retrospect, that the exposure of the patient to the severe cold at times during the healing process may have been the inciting cause for this condition.

Adrenalin shock following the injection of a local anesthetic containing epinephrine was never a problem as reported in previous expeditions. Two

cases of syncope occurred in the 250 anesthetic administrations but these could not be correlated with the increased blood epinephrine levels and were not reproduced in subsequent visits.

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BACTERIAL HYDROLYSIS OF CHONDROITIN SULFATE

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REPORTS of the presence of a mucopolysaccharide in human enamel and dentin are found in the literature dating from 1937; Meyer and Smyth,[1] Pincus,[2] Stack,[3] Hess and Lee,[4] Rogers,[5] and others have identified mucopolysaccharides in such materials. Sognnaes and Wislocki[6, 7] verified these findings with histochemical technics. Hess[4] and Pincus[2] identified the mucopolysaccharide as chondroitin sulfuric acid, a compound that has long been recognized as a constituent of cartilaginous tissues and bone matrix. Chondroitin sulfuric acid is a polymer consisting of a sulfate grouping, acetic acid, galactosamine, and glucuronic acid.

The first report of enzymatic breakdown of chondroitin sulfuric acid was made by Neuberg and co-workers.[8, 9] They inferred that the enzymatic reaction was one of depolymerization with hydrolysis of the sulfuric acid group. The crude enzyme employed in this investigation was an alcohol-ether dried preparation of a gram-negative rod of the Pseudomonas order. In a more recent investigation, Pincus[10] reported the sulfatase activity of a gram-negative rod on chondroitin sulfuric acid.

Schultz-Haudt and Scherp[11] have investigated the chondrosulfatase activity of microorganisms isolated from human gingival crevices. The hydrolysis of chondroitin sulfate was measured by a turbidimetric procedure. These investigators isolated and characterized the responsible microorganism as a micro-aerophilic diphtheroid bacillus. In the process of isolation and characterization of these organisms, a number of other types and combinations, including many strains of streptococci, failed to show chondrosulfatase activity.

The purpose of this investigation was to ascertain what action a crude enzyme preparation from bacteria associated with human dental caries might have on chondroitin sulfate. In view of recent investigations associating the streptococcus with dental caries,[12, 13] it was decided to investigate this group of bacteria for possible enzymatic activity.

MATERIALS AND METHODS

Preparation of the Enzyme.—Carious material was removed from the leading edges of lesions[13, 14] found in 10 patients in the clinic of Emory University School of Dentistry. After removal under the most aseptic conditions possible,

These studies were supported by grants from Bristol-Myers Products Division.
Received for publication Sept. 24, 1957; revised by authors May 5, 1958.
*Recipients of U. S. Public Health fellowships for dental students.

this material was incubated in pig heart infusion broth and differentiated on streak plates. Gram-stain and characteristic reactions when grown on blood agar, in methylene blue milk, in 6.5 per cent saline broth, and in bile salts, indicated that these organisms were identical to the "oral" streptococci described by Clarke,[14] Anderson and Rettger,[15] and Harrison.[13] A single colony was selected from a blood agar plate in order that a pure strain could be tested. To obtain the quantity of bacteria necessary, pure cultures of streptococci were grown in 3-liter batches of 0.5 per cent glucose-pig heart infusion broth for 24 hours. The bacteria were removed by centrifugation, washed with alcohol, followed by alcohol-ether (3:1) and pure ether, vacuum dried, ground to a fine powder, and stored over phosphorus pentoxide. From each 3-liter batch, approximately 0.5 Gm. of light gray powder (crude enzyme) was obtained.

Preparation of Substrate.—A commercial grade of crude chondroitin sulfate was purified by the method of Einbinder and Schubert.[16] Analyses of the purified chondroitin sulfate are given in Table I.

TABLE I
ANALYSIS OF PURIFIED SUBSTRATE*

CONSTITUENT	METHOD	THEORETICAL $C_{14}H_{19}NSO_4$	ANALYSIS
Hexosamine	Elson and Morgan[21]	29.49	28.4
Hexuronic acid	Dische[17, 18, 19]	31.96	29.2
Sulfate	Turbidimetric[20]	14.9	13.5
Total nitrogen	Micro-Kjeldahl	2.31	2.21
Moisture			6.78

*The material was soluble in water to the extent of 5 per cent, did not respond to Benedict's test for reducing sugar, formed no emulsion when a solution was shaken with chloroform and octanol, and no protein was detectable by measurement of the absorption at 280 mμ.

Preparation of the Digest Solution.—In Table II the standard conditions of the enzymatic digestions are outlined. In all digestions these materials remained the same with the exception of the variant indicated for each series. After the mixture of substrate, buffer, and activators was filtered through a fine sintered-glass funnel into sterile test tubes, the "enzyme" was added and a cotton plug inserted. The samples were digested at 37.5° C. in a constant temperature bath.

TABLE II
COMPOSITION OF DIGEST SOLUTIONS

"Enzyme"	50.0 mg. dried bacterial preparation
Substrate	5.0% potassium chondroitin sulfate (1.0% final concentration)
$MgCl_2$	0.001 M (final concentration)
Glutathione	10 mg.
Toluene	0.2 ml.
Tris (hydroxymethyl) aminomethane ("tris" buffer)	6-8 ml. (to make final volume)
Final volume	10.0 ml.
pH range	7.9-8.1

Method of Assay.—

1. *Hexosamine* was determined by the method of Elson and Morgan.[21] Reagent blanks were run with each determination and 1.0 ml. aliquots of the centrifuged and filtered digest were used as samples.

2. *Inorganic sulfate* was determined by a procedure involving barium iodate. An excess of barium iodate was added to the digest solution at zero time; after digestion 2 ml. aliquots of the centrifuged and filtered solutions were treated with potassium iodide and concentrated sulfuric acid. The liberated iodine was then back titrated with sodium thiosulfate. Results were always compared with a control digestion in which no enzyme was present. Inorganic sulfate was easily recovered by this procedure.

Series of Digestions.—After the enzymatic activity of the streptococcal preparation was established, further investigations were carried out along classical lines; the effects of time, of concentration of enzyme, of concentration of substrate, of activators and of inhibitors were studied.

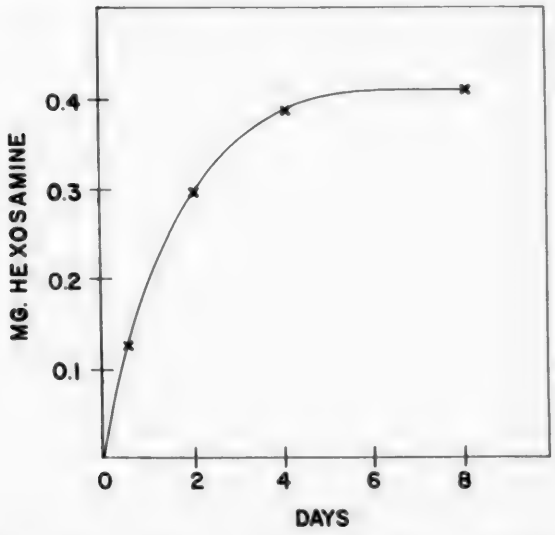

Fig. 1.—Effects of time on release of hexosamine (fixed concentration of enzyme and of substrate).

RESULTS

Sulfatase.—No sulfatase activity was detected in any of the experiments; no increase in free inorganic sulfate could be detected. All of the data cited are in terms of the hexosamine released by the bacterial enzymes.

Magnesium Ion and Glutathione.—Early in these studies it became apparent that magnesium ions and glutathione were essential for maximal activity. For example, with glutathione present at near optimal amounts the activity was increased twentyfold by the addition of magnesium ion; with magnesium ion

present at near optimal amounts the activity was increased approximately threefold by the addition of glutathione. Either constituent alone gave no or only minimal effects.

Time Activity Relation (Fig. 1).—The effects of time are reported in Fig. 1. The maximum splitting shown was only 2.5 per cent in 3 days under the conditions of Fig. 1; however, in comparing our results with those of Neuberg,[8] it should be noted that Neuberg used a much greater ratio of enzyme to substrate than was employed in this investigation. For comparison, a series of digestions was run under the conditions employed by Neuberg, and the maximum splitting was found to be 26.8 per cent in 72 hours.

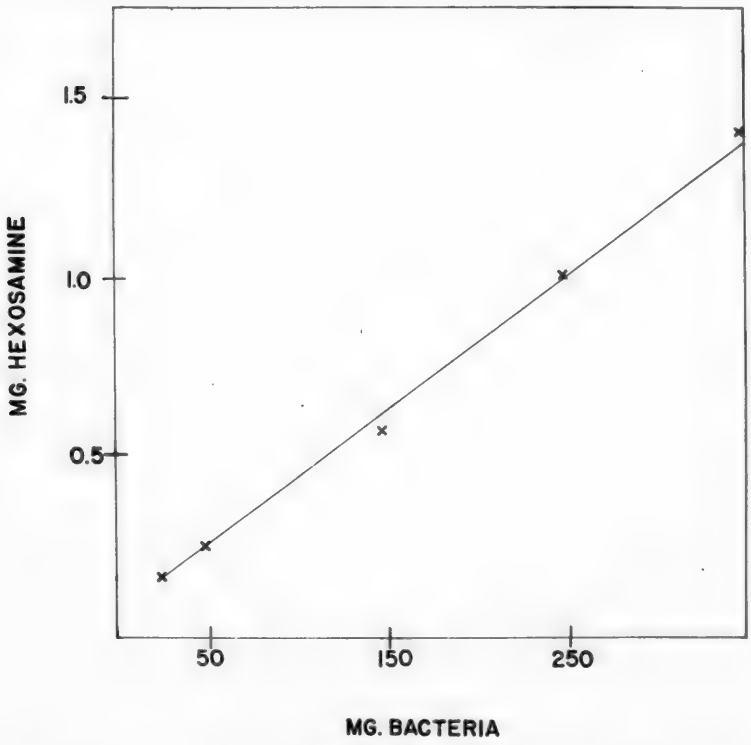

Fig. 2.—Effects of concentration of enzyme on activity (fixed concentration of substrate and of time).

Enzyme Concentration (Fig. 2).—The activity in the range of enzyme employed is directly proportional to the enzyme concentration. The straight line relation is typical of enzymatic reactions.

Glutathione Concentration (Fig. 3).—Increasing the concentration of glutathione up to 10 mg. per digest tube resulted in a marked acceleration of the enzymatic activity. Above this concentration no increase in activity was noted.

Substrate Concentration (Fig. 4).—A rapid increase in the rate of enzymatic cleavage was noted as the concentration of substrate was increased. At higher concentrations, the rate of increase was sharply diminished indicating that a point of enzyme saturation was approached at approximately 3.0 per cent concentration of substrate.

Effects of Inhibitors.—A number of inhibitors were tested and, in Table III, certain of the results are summarized; a great number of ineffective inhibitors are not included in this list. It is of interest that N-ethylmaleimide is a very potent inhibitor with only 1 to 5 parts per million required for half

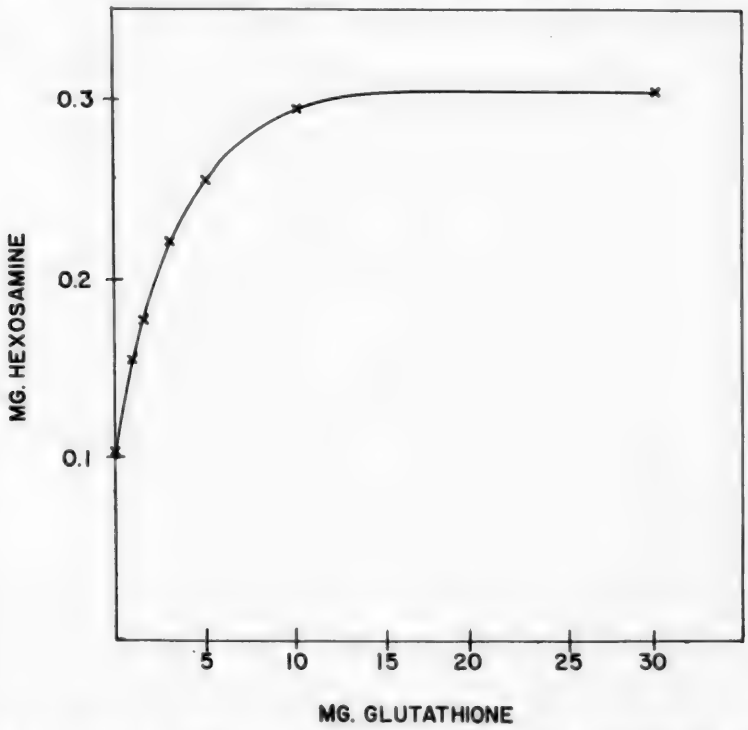

Fig. 3.—Effects of concentration of glutathione on release of hexosamine (fixed substrate, enzyme and time).

TABLE III
EFFECT OF INHIBITORS*

INHIBITOR	PPM REQUIRED HALF-INHIBITION
N-ethylmaleimide	1-5
Sodium fluoride	10-15
Cupric chloride	10-15
Silver nitrate	15-20
Lysine	20-30
Sarcosine	20-30
Urea	25-50

*A group of inhibitors were tested at various concentrations (in parts per million) under standard conditions. The results are expressed as ppm required for half-inhibition.

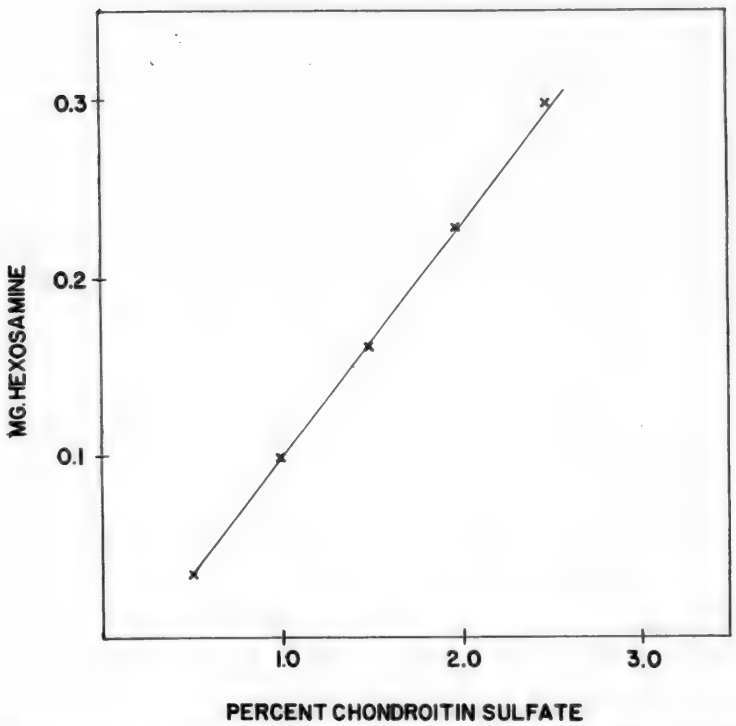

Fig. 4.—Effects of concentration of substrate on release of hexosamine (fixed enzyme and time).

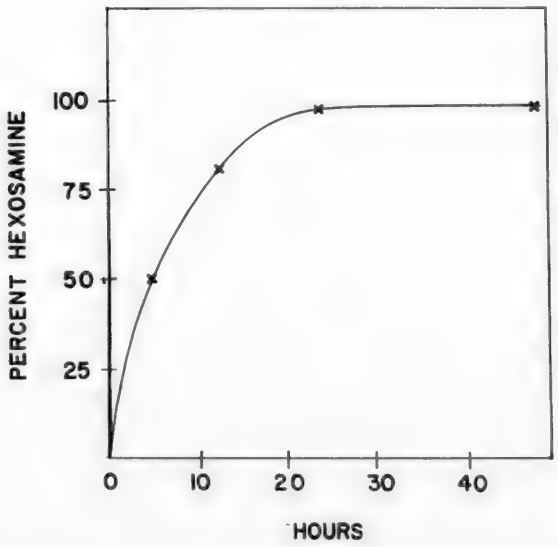

Fig. 5.—Hydrolysis of enamel mucopolysaccharide by bacterial enzyme. Results expressed as percentage of total hexosamine present.

inhibition. Metal ions such as copper and silver were inhibitory at reasonable levels and fluoride ion was also effective. A large number of amino acids and related compounds were tested; results with urea, lysine, and sarcosine are given because these compounds appear to inhibit at reasonable levels.

Hydrolysis of Enamel Chondroitin Sulfate.—Cattle teeth were cleaned and pulverized and the enamel, dentin and cementum were separated by the centrifugal method of Manly and Hodge.[22] The enamel was decalcified by dialysis with hydrochloric acid and, finally, the crude organic material was precipitated with ethanol. This material had a nitrogen content of 11.3 per cent, an ash content of 0.0 per cent, and a content of hydroxyproline of 8.4 per cent. The apparent content of hexosamine in a hydrolysate prepared with 6 N hydrochloric acid was 1.7 per cent. This crude material, containing both chondroitin sulfate and protein, was used as a substrate for the bacterial enzyme. As is summarized in Fig. 5, the total hexosamine was quickly released. Thus, the chondroitin sulfate from enamel appears to be more susceptible to hydrolysis by the bacterial enzymes than the purified commercial material.

DISCUSSION

In consideration of the presence of chondroitin sulfate in dental hard tissues, an explanation of the etiology of initial carious penetration is suggested. The strong resistance of enamel and dentinal protein (eukeratin) to proteolytic digestion[23] makes it reasonable to think that some other grouping in the protein molecules (either the soluble fraction or the polypeptide fraction)[24] provides a point of attack. Inasmuch as bacteria commonly found in caries are capable of destroying the mucopolysaccharides of teeth, it is reasonable to suggest that this destruction is a primary reaction in the formation of caries.

SUMMARY

Decomposition of the purified mucopolysaccharide, chondroitin sulfuric acid, by a crude enzyme preparation of bacteria of carious origin has been investigated. Data are presented establishing enzymatic depolymerization with the release of hexosamine. This depolymerization was investigated under a variety of conditions. Magnesium ion and glutathione were required for optimal activity. A variety of inhibitors were effective; the most potent inhibitor encountered was N-ethylmaleimide.

The enzyme was effective in the hydrolysis of the mucopolysaccharide of enamel from the teeth of cattle and, in fact, was considerably more effective than in the hydrolysis of purified chondroitin sulfate of commercial origin.

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CALCIFICATION

XVI. Composition of Bones and Teeth in Relation to Blood and Diet in the Cotton Rat

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PREVIOUS work supports the hypothesis that there is . . . "a relationship between the inorganic composition of teeth and the fluid from which the tooth salts precipitate and that the composition of this fluid in turn is related to the blood serum."[1-3] This work was partially based on analyses of rat incisors, which are in relatively rapid equilibrium with body fluids.[1] Molars, however, have been demonstrated to be more static organs;[4-5] the rate of turnover of Ca^{45} and P^{32} fed to albino rats being about four times slower for the dentin and about sixteen times slower for the enamel than for the corresponding incisor components.[6] It was thus decided to investigate whether the above hypothesis was applicable to molars. If changes could be induced in the composition of molars, our concepts relating composition to caries susceptibility could be tested.[1, 2, 7-10]

While molars were of primary interest in providing an experimental background for evaluating the caries susceptibility concept, the composition of rapidly growing incisors and the 2 typical long bones (tibia and femur) were also studied to understand further the relationship of these tissues to each other. The cotton rat was chosen as the experimental animal because of its caries susceptibility on high sugar diets.[11] As a control, corresponding studies of the Wistar rat were undertaken.

EXPERIMENTAL

Cotton Rat Colony.—Stock cotton rats, obtained from the Public Health Service of Lansing, Michigan, were kept on a mixture of Pratt's pellets and Bill's stock diet[12] to maintain the offspring on minimal sources of vitamin D. The third generation was sufficiently tame to be weighed without damage.

Preliminary Experiments.—To properly correlate the studies of the cotton rat with earlier composition studies of the Wistar rat,[1] the 2 species were compared under the new experimental conditions. The diets in these experiments

A preliminary report of this work was presented in part before (1) the Division of Biological Chemistry at the 121st Meeting of the American Chemical Society, Milwaukee, March 30—April 3, 1952, and (2) the Division of Biological Chemistry at the 124th Meeting of the American Chemical Society, Chicago, September 6-11, 1953.

These studies were supported in part by: (1) a grant from the National Institutes of Health, (D-55), U. S. P. H. S., and (2) a contract between the Office of Naval Research, Department of the Navy, and the Jewish Hospital of Brooklyn (NR 180 025). Early phases of the work were aided by funds from the Williams-Waterman Foundation, New York, N. Y.

Received for publication Oct. 21, 1957; revised by authors April 16, 1958.

contained liver and yeast supplements instead of the yeast used earlier. The additional incorporation of 0.1 per cent calcium (as 0.25 per cent $CaCO_3$) in the low calcium-high phosphate diet of the cotton rat was needed to prevent tetany. An age factor was also introduced, since the cotton rats placed on the diet were 16 days old to allow changes to be induced in the molars, while the Wistar rats were started at 23 days of age.[1]

The effect of the liver-supplemented diet and the age factor was determined by placing litters of cotton rats, 16 and 21 days of age, as well as litters of Wistar rats of corresponding ages, on diets with yeast or liver supplements. The high calcium-low phosphate diet was employed and the $PO_4:2CO_3$ ratio* was determined by methods previously described.[1, 15-18] The results (Table I) showed that neither age nor dietary supplement was responsible for the major differences in bone and teeth composition.

TABLE I

WISTAR VS. COTTON RAT—COMPOSITION ($PO_4:2CO_3$) (MEANS)*

ANIMAL	AGE AT WEANING (DAYS)	DIET†	UPPER INCISOR		UPPER MOLAR		TIBIA	FEMUR
			ENAMEL	DENTIN	ENAMEL	DENTIN		
Wistar‡	16	B-I Liver	4.28	3.50	4.22	3.46	2.36	2.37
Wistar‡	16	B-I Yeast	4.12	2.79	4.07	3.19	2.29	1.78
Wistar‡	21	B-I Liver	4.08	3.65	3.42	2.58	2.52	1.74
Wistar‡	21	B-I Yeast	2.53	3.53	3.74	3.09	2.44	2.19
Cotton§	16	B-I Liver	7.19	4.27	5.64	3.65	3.26	2.85
Cotton‖	16	B-I Yeast	4.30	3.17	5.69	3.51	3.17	2.94
Cotton‡	21	B-I Liver	5.75	4.02	5.21	4.84	3.06	2.22
Cotton‡	21	B-I Yeast	6.07	6.13	7.71	4.76	3.47	3.91

*All ratios are molar. The $PO_4:2CO_3$ ratio is represented by n in the composition formula $Ca_3(PO_4)_2$ n $CaCO_3$ in which approximately 6 per cent calcium is replaced by Mg, Na, Sr, Pb, etc., and some CO_3 is replaced by citrate, PO_4, OH, flouride, etc.

†Diet B-I (Table II) was used, except that yeast was substituted for the liver extract in one of each pair. The yeast was added in such proportions that the calcium and phosphorus content of the diet was kept constant.

‡Three animals.

§Four animals.

‖Five animals.

Final Experiments.—Six litters of cotton rats were chosen for a first experiment, and another 6 litters were chosen for a second experiment. The animals were weighed weekly in the first study and daily in the second. The litters were divided so that littermates were placed on the high calcium-low phosphorus diet, the low calcium-high phosphorus diet, and the stock diet for 28 days. All diets (Table II) were supplemented with liver and mineral additives, with the exception of the stock diet. After the experimental period, the animals were sacrificed by severing the jugular vein and carotid arteries. The blood, tibias, femurs, lower and upper incisors and molars (both dentin and enamel) were analyzed for carbonate, calcium, and phosphate by methods previously described.[1, 15-18] The 2 upper incisors were pooled as were the 2

*The *$PO_4:2CO_3$ ratio is equal to "n"* is the usual way of representing the composition formula for bone and teeth, $Ca_3(PO_4)$ n $(CACO_3)$, where approximately 6 per cent of Ca is replaced by Mg, Na, Sr, Pb, etc., and some CO_3 is replaced by citrate, PO_4 OH, fluoride, etc. [1, 8, 13, 14]

TABLE II

COMPOSITION OF DIETS

DIETS	FOOD COMPONENTS	GRAMS	MINERAL COMPONENTS	GRAMS	VITAMINS (MG.)	Ca (%)	P (%)	Ca: P
Basal I	Yellow corn meal (degerminated)	75	Salt mixture*	2.36	240.0 Vitamin mixture*	0.286	0.118	0.242
	Wheat gluten	18						
	Liver extract (1:20)	1.5						
B-I High Ca Low P	Basal I	97	$CaCO_3$	3.0		1.23	0.118	10.4
C-I Low Ca High P	Basal I	96	Na_2HPO_4	3.0		0.10	0.81	0.123
			$CaCO_3$	0.18				

*Composition of Salt Mixture and Vitamin Mixture.

Salt Mixture

Components	Grams
NaCl	0.84
KCl	0.72
$MgSO_4 \cdot 7H_2O$	0.53
$Fe(C_6H_5O_7) \cdot 6H_2O$	0.15
$MnSO_4 \cdot 2H_2O$	0.03
$ZnCl_2$	0.01
$CuSO_4 \cdot 5H_2O$	0.03
KI	0.05

Vitamin Mixture

Components	Mg.
Thiamine	0.25
Riboflavin	0.30
Nicotinic acid	2.50
Pyridoxine	0.25
Calcium pantothenate	2.00
Choline HCl	100.00
Inositol	100.00
p-amino benzoic acid	30.00

lower incisors, the 6 upper molars, and the 6 lower molars. Only 1 tibia and 1 femur from each animal were analyzed. The results are given in Tables III and IV.

TABLE III

PER CENT COMPOSITION OF TEETH AND BONE IN RELATION TO BLOOD AND DIET IN THE COTTON RAT

	DIET B-I			DIET C-I			STOCK DIET		
	CO_2	Ca	P	CO_2	Ca	P	CO_2	Ca	P
Serum (mg. %)	49.1*	11.9	3.6	56.1*	6.3	11.0	54.1*	11.0	9.4
Lower molar									
Enamel (%)	3.73	35.1	17.5	2.84	35.4	18.2	3.53	32.9	16.4
Dentin (%)	4.25	28.2	14.1	2.93	26.6	13.9	2.65	27.0	13.4
Upper molar									
Enamel (%)	4.06	31.3	15.6	2.80	35.5	18.4	3.57	34.7	17.7
Dentin (%)	3.40	24.6	12.4	2.82	27.4	14.0	3.00	25.8	12.9
Lower incisor									
Enamel (%)	3.86	32.1	16.2	2.72	36.8	20.0	3.52	36.0	17.5
Dentin (%)	3.31	25.8	13.7	2.72	24.0	13.8	2.79	27.0	14.2
Upper incisor									
Enamel (%)	4.23	38.4	19.5	2.67	35.0	18.9	3.34	36.4	19.1
Dentin (%)	3.26	20.9	13.1	2.83	27.2	13.6	2.78	24.1	14.2
Tibia (%)	3.22	17.4	8.70	2.58	19.0	10.7	3.25	20.9	11.1
Femur (%)	3.20	15.7	7.85	2.54	17.2	9.40	3.25	20.1	10.6
Dietary Ca:P (% ratios)		10.4			0.123				

*Serum CO_2 is expressed as vol. % CO_2.

The statistical significance of the results was evaluated by the methods outlined by Fisher[19] and are presented in Table V. The "P" values represent the probability that differences are due to chance.

TABLE IV

COMPOSITION OF TEETH AND BONE IN RELATION TO BLOOD AND DIET IN THE COTTON RAT*

	DIET B-I		DIET C-I		STOCK DIET	
	$PO_4:2CO_3$	$Ca:PO_4$	$PO_4:2CO_3$	$Ca:PO_4$	$PO_4:2CO_3$	$Ca:PO_4$
Serum	26.3†	2.57	71.5†	0.47	61.5	0.90
Lower molar						
Enamel	4.60	1.54	6.20	1.51	5.01	1.54
Dentin	3.97	1.56	4.60	1.49	4.90	1.56
Upper molar						
Enamel	3.79	1.56	6.52	1.50	5.07	1.52
Dentin	3.61	1.54	4.86	1.52	4.20	1.55
Lower incisor						
Enamel	4.36	1.52	7.34	1.44	5.61	1.61
Dentin	4.01	1.47	4.95	1.35	5.07	1.48
Upper incisor						
Enamel	4.33	1.54	6.46	1.49	5.69	1.46
Dentin	3.94	1.47	4.70	1.38	5.04	1.32
Femur	2.40	1.56	3.73	1.40	3.15	1.47
Tibia	2.68	1.55	4.21	1.37	3.30	1.47
Dietary Ca:P (% ratios)	10.4		0.123			

*Same as first footnote in Table I.
†Serum $PO_4:2CO_3$ values are 1,000 times those actually obtained

TABLE V

STATISTICAL SIGNIFICANCE OF THE DIFFERENCE OF MEANS ("P" VALUES)*

	$PO_4:2CO_3$			$Ca:PO_4$		
	B-I vs. C-I	B-I vs. STOCK	C-I vs. STOCK	B-I vs. C-I	B-I vs. STOCK	C-I vs. STOCK
Lower incisor						
Enamel	>0.01	>0.01	>0.01	>0.03	>0.01	>0.01
Dentin	>0.05	>0.03	0.2	>0.02	0.8	>0.01
Upper incisor						
Enamel	>0.01	>0.02	>0.02	>0.05	>0.03	0.5
Dentin	>0.04	>0.01	>0.05	>0.02	>0.01	0.1
Lower molar						
Enamel	>0.01	>0.05	>0.01	0.1	1	0.6
Dentin	>0.02	>0.02	>0.05	>0.05	1	>0.05
Upper molar						
Enamel	>0.01	>0.01	>0.01	>0.05	0.5	0.8
Dentin	>0.02	>0.03	>0.05	0.7	0.9	0.7
Tibia	>0.01	>0.04	>0.04	>0.01	>0.04	>0.02
Femur	>0.01	>0.05	>0.05	>0.05	>0.04	>0.03

*A "P" value of 0.05 or less indicates that the differences between two means is of statistical significance and that the probability that the difference between the means is due to chance is five parts or less per 100.

RESULTS

Weight Changes.—The mean weight gain of animals on the high calcium-low phosphorus diet was 18.6 grams, of animals on the low calcium-high phosphorus diet 23.4 grams, and of animals on the stock diet, 40.7 grams.

Blood Composition.—For animals on the high calcium-low phosphorus diet, the serum calcium levels tended to be normal and the phosphate levels low, whereas for those on the low calcium-high phosphorus diet the serum calcium was low and the phosphate somewhat above normal. The serum calcium and phosphate levels for the animals on the stock diet were normal, the calcium being within the range found on the high calcium diet, and the phosphate being somewhat lower than on the high phosphorus diet. The total serum carbonate appeared to be the same regardless of the dietary group (see Tables III and IV).

Bone Composition.—The percentages of carbonate, calcium, and phosphate, expressed as P, are given in Table III. The ash was highest in the enamel, lower in the dentin, and lowest in the bone.

Composition of Bone in Relation to Blood.—The $PO_4:2CO_3$ and $Ca:PO_4$ ratios of the tibias and femurs are related to the corresponding serum ratios (Table IV). As previously shown in the Wistar rat,[20, 21] these serum ratios are related to dietary Ca : P ratios. Compared to the Wistar rat, the bone $PO_4:2CO_3$ ratios are higher and the $Ca:PO_4$ ratios are lower in the cotton rat under presumably similar conditions.

Composition of Incisors in Relation to Blood.—The relationships between the $PO_4:2CO_3$ ratios of the blood serum and both the enamel and dentin are evident both in the lower and upper incisors (Table IV). The influence of the blood $PO_4:2CO_3$ ratio appeared greater on the enamel than on the corresponding dentin, as previously noted in the Wistar rat.[1] In contrast to observations on the Wistar rat, the $PO_4:2CO_3$ ratio of the enamel is higher than that of the dentin, as is the case for human teeth.[20]

$Ca:PO_4$ ratios of the enamel and dentin are only mildly influenced by the serum $Ca:PO_4$ ratios, as in the Wistar rat.[1] The differences between the enamel and dentin, marked in the Wistar rat, are minimal in the case of the cotton rat. In the latter, the ratios are much closer to each other, resembling those of human enamel and dentin.[22] This further indicates the value of the cotton rat in the experimental study of caries.

Composition of Molars in Relation to Blood.—The composition of the molars in relation to blood and diet is presented in Table III. In spite of the partial mineralization prior to placement on the experimental diet, distinct relationships can be seen between the $PO_4:2CO_3$ ratios of the molars and those of blood and diet. The influence is greater on the enamel than on the dentin. The $PO_4:2CO_3$ ratios are higher in enamel than in dentin (just as in human teeth), especially in animals on the low calcium-high phosphorus diet. The results imply that the differences in the $PO_4:2CO_3$ ratios of enamel and dentin may depend, at least in part, on the type of diet administered. In contrast to the results obtained for bone, the $Ca:PO_4$ ratios do not seem to be significantly related to serum or dietary Ca : P ratios.

DISCUSSION

It is evident from these studies that composition of growing molars can be influenced by diet. The $PO_4:2CO_3$ ratios of molars are related to those of diet (Tables III, IV). These changes in composition are understated, since composition of the portion of the molars already mineralized at the beginning of the experiment can be modified only to a slight degree as indicated by turnover studies,[4-6] while that of bone and continually growing incisors can be markedly influenced.[1-3, 7, 8, 20] The first molars are almost completely mineralized and the second molars are partially mineralized at 16 days of age, while the third molars are mineralized for the most part during the experimental period.

The $PO_4:2CO_3$ ratios of the tibia, femur, enamel, and dentin of the incisors and molars, are related to the $PO_4:2CO_3$ ratios of blood serum. Although $PO_4:2CO_3$ ratios of bone, enamel, and dentin are related to that of blood, they are different for each of these tissues; enamel having the highest ratio and bone the lowest. In Table IV, the relationship between the $PO_4:2CO_3$ ratios of the mineral tissues of the cotton rat and the blood serum is clearly expressed. Another important difference between these three tissues is in the density of mineralization as measured by total per cent composition of carbonate, calcium, and phosphate. The density is highest in enamel, next in dentin, and lowest in bone. Another difference is the significant influence of diet on bone and dentin $Ca:PO_4$ ratios, as compared with the minor influence on $Ca:PO_4$ ratios of enamel.

Differences in the function of the "local factor(s)," as defined by heredity, are manifested when the present analytical results are compared to those obtained on the Wistar rat.[1, 20, 21] Given similar dietary Ca:P ratios, the incisor and bone $PO_4:2CO_3$ ratios are lower and the $Ca:Po_4$ ratios are higher in the Wistar rat than in the cotton rat. That these differences are not due to age or altered dietary supplements is indicated in Table II.

Differences found in comparing the present studies of the cotton rat with earlier studies of the Wistar rat appear generally to be due to hereditary species differences in the functioning of the "local factor(s)." Any differences in the "local factor(s)," resulting in a higher $CO_3:PO_4$ ratio in mineralized tissues, would tend to increase caries susceptibility. This is because teeth with higher $CO_3:PO_4$ ratios are more caries susceptible, as predicted from differences in chemical properties.[1, 8-10] In addition, hereditary factors affecting the relationship between diet and serum $CO_3:PO_4$ ratios of blood would, in turn, influence the composition of mineralized tissues.[1-3, 20, 21] It can be postulated, therefore, that caries susceptibility is influenced not only by environmental factors which influence tooth composition, but also by hereditary differences in the "local factor(s)" of calcification.

SUMMARY

The composition of bones and teeth, including the molars, is related to the composition of the blood serum in the cotton rat.

The differences in the composition of enamel, dentin, and bone indicate differences in the "local factor(s)" of calcification.

The differences in the composition of bone and teeth between the Wistar rat and cotton rat imply the existence of hereditary differences in the "local factor(s)."

The authors are indebted to Professor James H. Shaw of the Harvard School of Dental Medicine for his invaluable advice on the maintenance of the cotton rat and for reviewing the manuscript.

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ELECTRON MICROSCOPIC OBSERVATIONS OF THE DIFFERENCES IN THE EFFECTS OF STANNOUS FLUORIDE AND SODIUM FLUORIDE ON DENTAL ENAMEL

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CLINICAL tests have shown that topical application of fluorides to erupted teeth will reduce dental caries.[1] Stannous fluoride has been found to be more effective than sodium fluoride in reducing decay when applied topically.[2, 3, 4]

In an attempt to elucidate the mechanisms by which the fluoride exerts its protective action, extensive studies[5, 6, 7] have been made of the effects of inorganic salts on rate of solution of enamel in acid solutions. Since many salts, and particularly the fluorides, reduce the solution rate, studies of the nature of the chemical and physical changes which occur in the enamel have been made using electron microscopy and x-ray and electron diffraction.[8-13] It has been shown that treatment of enamel with sodium fluoride results in the formation of calcium fluoride. The diffraction studies have shown no such changes after treatment with stannous fluoride, but have suggested the presence on the enamel surface of an amorphous layer of unknown composition. The prior work does not include a comparative study of the visible effects of the reagents on the structure and properties of intact enamel surfaces.

The present work shows the effects on whole enamel surfaces of treatments with aqueous solutions of stannous fluoride, sodium fluoride, and non-fluoride stannous salts, over a range of pH values. In addition, the effects resulting from the exposure of these treated surfaces to acid buffer solutions are described.

EXPERIMENTAL METHOD

The teeth used in this investigation were human maxillary first incisors which were stored in a 0.5 per cent formaldehyde solution. No prior history of the teeth was available. Rectangular pieces of whole enamel from these teeth were cut from the labial surface. The area of the enamel surface varied between 4 and 16 sq. mm., and generally it was possible to cut 9 such specimens from a single tooth. The cutting was done with a circular jeweler's slotting high-speed steel saw operated at low speeds under water to prevent heating and drying of the tooth. For convenience in handling, the enamel

Presented in part at the 34th General Meeting of the International Association for Dental Research, St. Louis, Missouri, March 22-25, 1956.

Received for publication Oct. 24, 1957; revised by authors May 15, 1958.

pieces were mounted in a plastic cement* on the ends of ¼ inch diameter plastic rods. All si es of the specimens except the labial surfaces were covered with the cement. The enamel surface was then polished to a high finish, under light pressure, against a rotating wheel covered with a wet billiard felt which was impregnated with appropriate abrasive powder. The over-all contour of the enamel surface was maintained, and only enough enamel was removed to produce a highly polished surface free from the natural microscopic surface imperfections. Two or 3 different silicon carbide abrasives in a graded series were used and were followed by a final polishing with gamma alumina.† The appearance of the enamel surface was constantly checked with a metallurgical microscope at magnifications up to 500 times.

After polishing, the enamel samples were treated as desired at room temperature under a standard set of conditions. The enamel mounted on the plastic rod was held in a set quantity of the desired solution (75 ml. in a 100 ml. beaker). The solution was agitated by a propeller stirrer turning at 500 r.p.m. Each treatment was followed by a distilled water rinse. All solutions were made up by weight. The pH was measured with a Beckman Model G pH meter. The stannous fluoride,‡ sodium fluoride§ and stannous chloride§ samples were obtained from commercial sources. The stannous perchlorate and sulfate samples were prepared in our laboratories. All samples showed cation and anion analyses within 5 per cent of the theoretical values.

Metal replicas were made of the enamel surfaces for examination with the electron microscope. This was done by placing the enamel sample, still cemented to the plastic rod, in a vacuum evaporator and evaporating chromium onto the enamel surface at approximately a 30-degree angle. The thickness of the chromium deposited on the enamel, selected empirically for good contrast, was in the range of 150 Å normal to the surface. The thin metal replica was then reinforced by evaporating a layer of carbon on top of the chromium. The additional film of carbon, while giving added thickness and strength to the replica, did not appreciably reduce the transparency to electrons.

To remove the metal replica, the enamel was sawed from the plastic rod and placed in a small dish containing 1 N hydrochloric acid. When care was taken not to wet the top surface, the replica floated free in a few minutes while the enamel sample settled to the bottom. The replica floating on the surface was picked up on a specimen screen, rinsed in distilled water, air dried, and examined in the RCA EMU-2D electron microscope. All the electron micrographs presented in this paper were taken at 3,000× and are marked with a micron scale for easy reference. Because of dehydration during the vacuum evaporation and attack by the acid during removal of the replica, the enamel samples could be used only once.

*F-88 made by American Consolidated Dental Co., and Dura Base made by Reliance Dental Mfg. Co.

†Microid Gamma Polishing Alumina, Griffin & Tatlock Ltd., England.

‡Metal & Thermit Corporation.

§J. T. Baker Chemical Co. Reagent.

To keep enamel sample differences to a minimum, most comparisons were ultimately made on the several enamel samples obtained from the same tooth. Comparisons among enamel samples from different teeth were made by running identical tests on 1 or 2 samples from each tooth. In the final analysis, these differences among samples can only be compensated for by doing several replicate experiments. Replicas were made from nearly 500 enamel samples in the course of this study, and roughly 10 repeat experiments were made for each typical result shown in this paper.

EXPERIMENTAL RESULTS

Polished and Acid-Etched Enamel.—The starting point of all the experiments was polished enamel. For orientation, a progression of magnifications of such a surface is shown in Figs. 1-3 beginning with light micrographs taken with a metallurgical microscope at 100× and 400×, and ending at 3,000× taken with the electron microscope. At the higher magnifications the enamel rod outlines could be more clearly seen. However, more details of the rod structure became visible when the polished enamel had been exposed for 5 minutes to 0.1 N lactic acid adjusted to pH 5.0 with NaOH (Fig. 4). As has been shown by others, the mineral portion within the rod centers dissolved away at a more rapid rate than the enamel rod sheath. These conditions of acid exposure were used throughout this study as a means of demonstrating a typical effect of an acid solution on the enamel surfaces. Variations of 3 to 6 in the pH of the buffer and of 1 to 15 minutes in the time of exposure appeared to alter the rate rather than the qualitative nature of the acid attack.

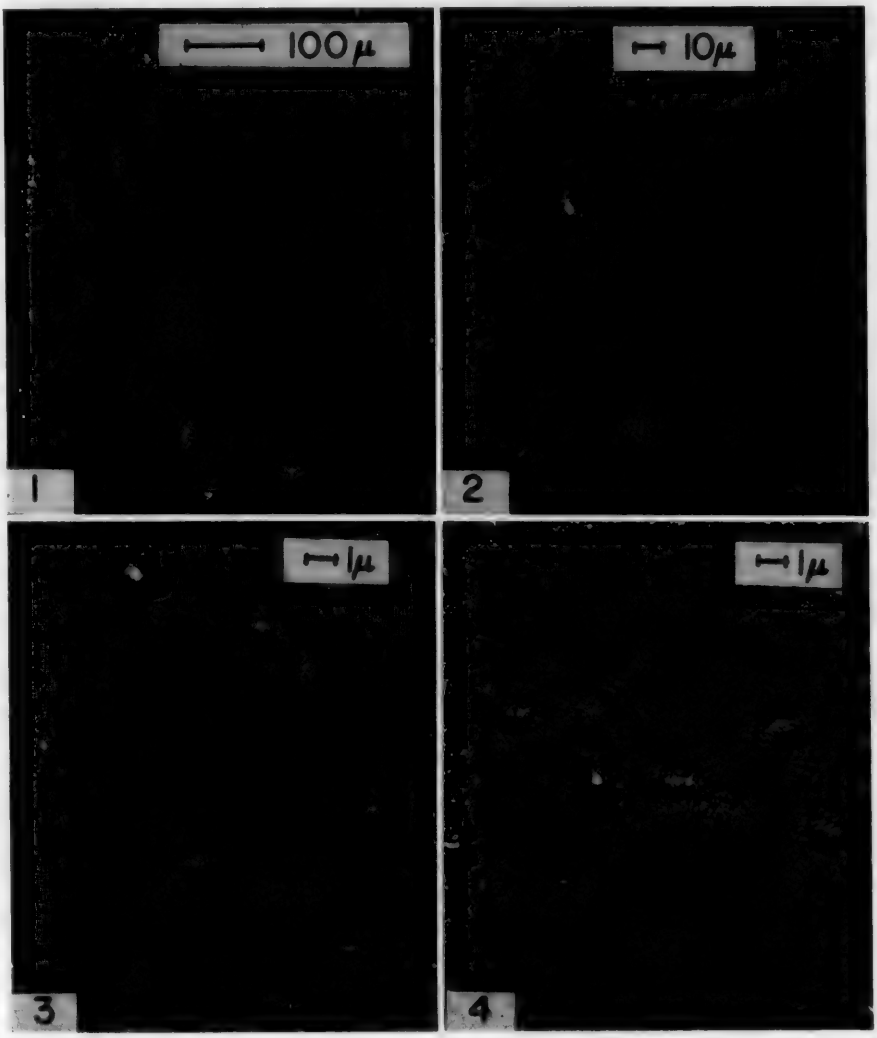
Stannous Fluoride-Treated Enamel.—Enamel was treated with stannous fluoride solutions at different pH values, and the enamel surface was examined. This was followed by exposing SnF_2-treated enamel to lactic acid buffer to determine the effect of acid on these surfaces. As mentioned previously, an enamel sample could be used only once for electron microscopic observation. This necessitated the use of separate enamel specimens for studying the effect of SnF_2 treatment, and the subsequent acid exposure.

In the first series, enamel was treated for 5 minutes with a 0.4 per cent stannous fluoride solution, which provided about 3,000 ppm of stannous ions and 1,000 ppm of fluoride ions. The solution was used at the natural pH of 3 and also at higher pH values after adjusting with sodium hydroxide. Where precipitation occurred, the enamel was treated with the complete slurry. No change of any kind could be found in the surfaces treated with the stannous fluoride solutions at pH values between 3 and 7. This is illustrated in Figs. 5, 6 and 7, showing treatments at pH 3.0, 5.0, and 7.0. These results are particularly striking when compared with the change produced by lactic acid at pH 5.0 (Fig. 4).

The next pair of electron micrographs (Figs. 8 and 9) show the results of exposure of stannous fluoride-treated enamel to the lactic acid buffer at pH 5.0 for 5 minutes. When enamel was treated with stannous fluoride at pH values between 3 and 7, the lactic acid buffer did not visibly etch the

enamel even though this same acid exposure severely etched the untreated enamel. Clearly the stannous fluoride treatment increased the resistance of enamel to etching.

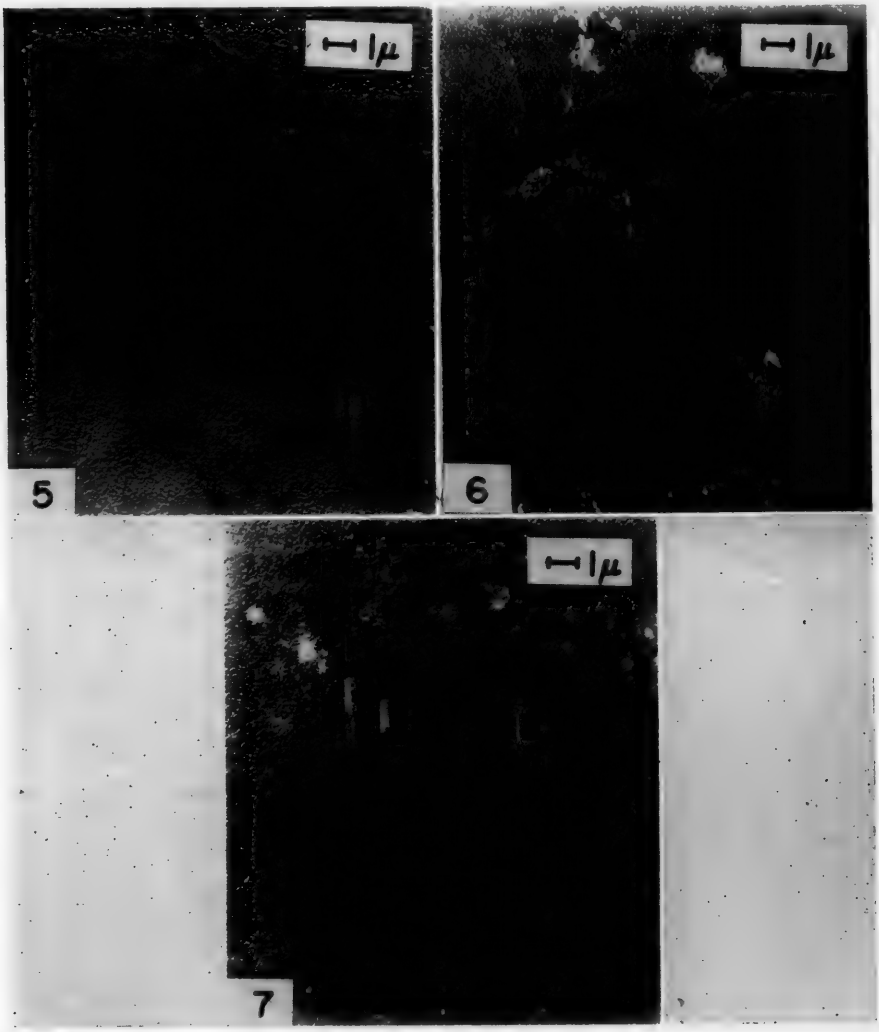
This protective effect of stannous fluoride treatment was also obtained with low concentrations. Fig. 10 shows an electronmicrograph of enamel treated with 0.02 per cent stannous fluoride (about 150 ppm Sn^{++}, and 50



Figs. 1-4.—Polished enamel: Fig. 1, orig. mag. ×100; Fig. 2, orig. mag. ×400; Fig. 3, orig. mag. ×3,000.

Increasing magnification of dental enamel surface relates the enamel rod structure observed at the various magnification levels. Figs. 1 and 2 are light micrographs taken directly of the enamel surface using top illumination. Fig. 3 is an electron micrograph of a metal replica of the enamel surface. Fig. 4 is an electron micrograph of polished enamel after exposure to 0.1 N lactic acid, pH 5.0, for 5 minutes. The centers of the enamel rods have been dissolved at a faster rate than the rod edges.

ppm F^-) for 5 minutes at pH 3.0, and Fig. 11 shows a similarly treated sample after subsequent exposure to the lactic acid buffer. The treatment reduced the effect of acid markedly and did not change the appearance of the enamel surface.

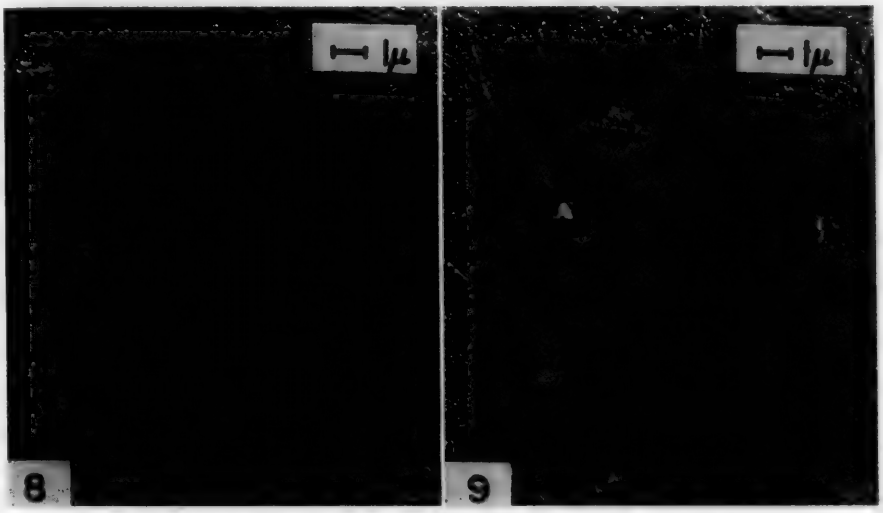


Figs. 5-7.—SnF_2 treatment: pH 3 (Fig. 5); pH 5 (Fig. 6); and pH 7 (Fig. 7). Treating polished dental enamel for 5 minutes with 0.4 per cent SnF_2 over a pH range of 3 to 7 caused no appreciable alteration from untreated polished enamel.

Sodium Fluoride-Treated Enamel.—Enamel was treated for 10 minutes with sodium fluoride over a pH range of 3 to 7 at a concentration of 0.2 per cent. This gave about 900 ppm F^-, approximately the same fluoride level as the previous stannous fluoride solutions. The pH was adjusted to various

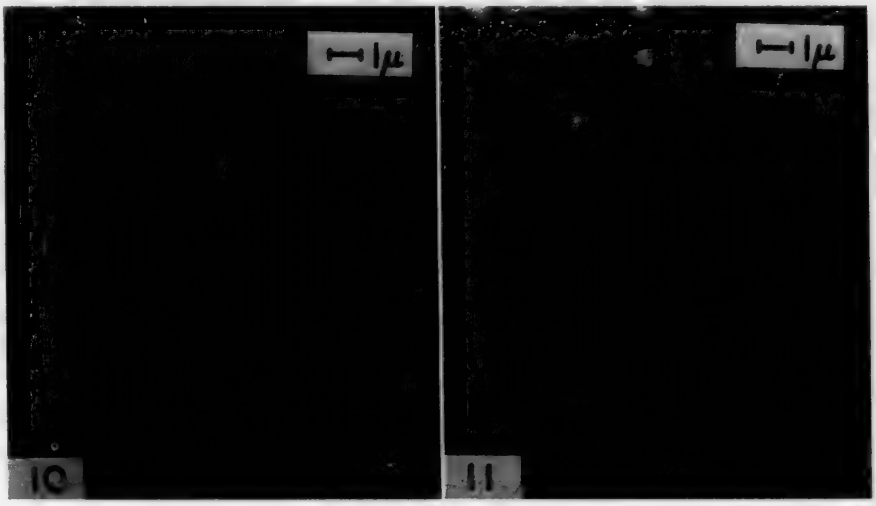
levels with hydrofluoric acid or sodium hydroxide. The additional fluoride increased the concentrations somewhat (a maximum of 5 per cent), but this seemed preferable to the introduction of another ion species.

At pH 3.0 (Fig. 12), the enamel surface was almost completely covered with a layer of small crystals which totally obliterated the original tooth



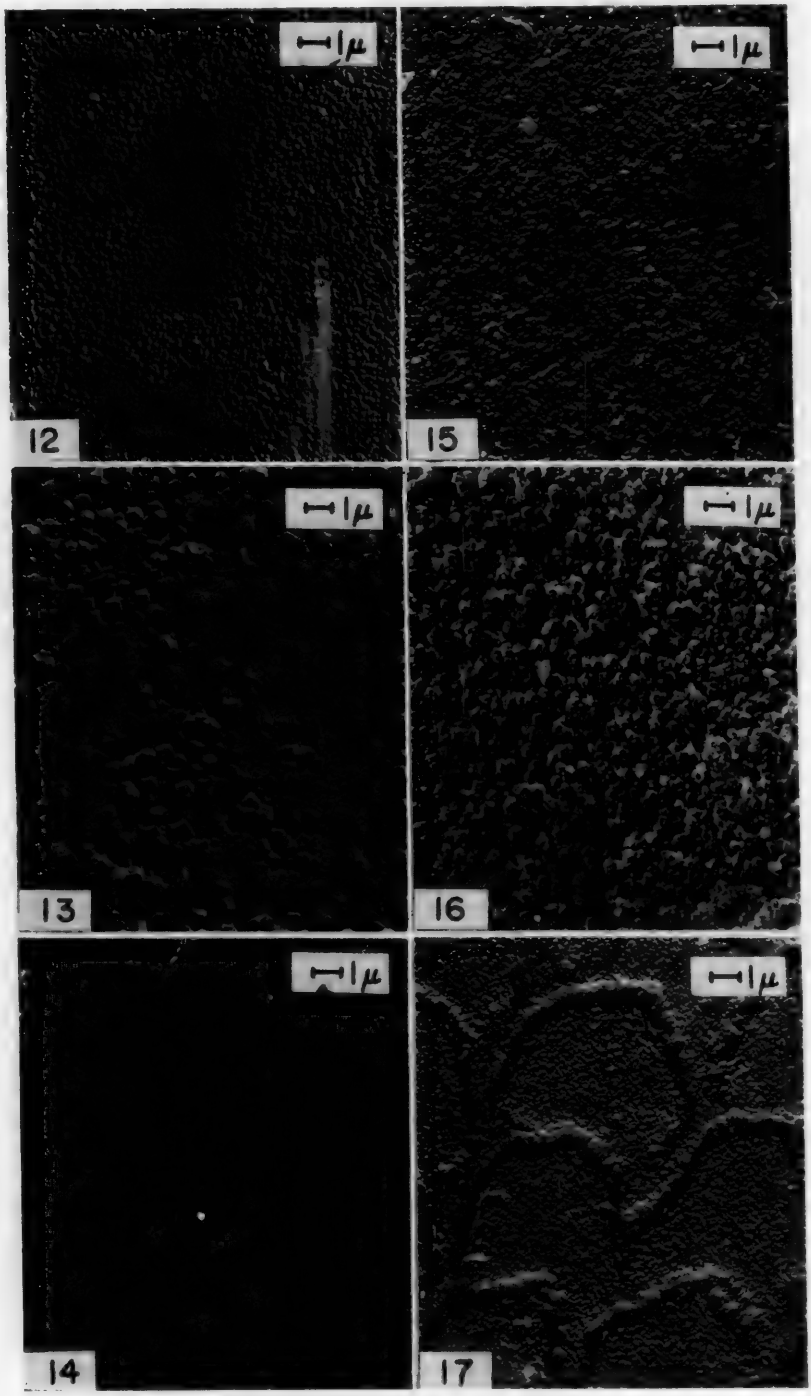
Figs. 8-9.—Treatment—SnF_2, pH 3, and exposed to lactic acid (Fig. 8). Treatment—SnF_2, pH 7, and exposed to lactic acid (Fig. 9).

Treating polished dental enamel for 5 minutes with SnF_2 solutions (pH 3 and 7) protected the enamel against attack by lactic acid exposure of 5 minutes at pH 5.0.



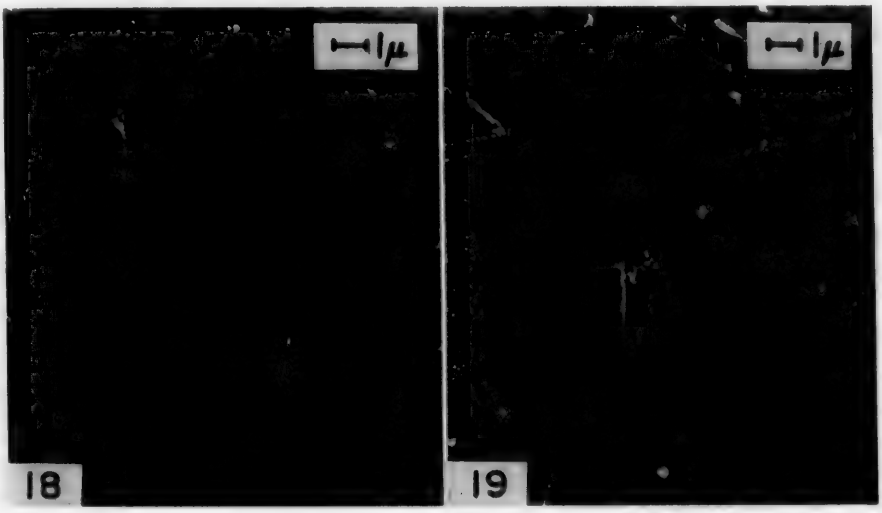
Figs. 10-11.—Treatment—0.02 per cent SnF_2 at pH 3.5 (Fig. 10). Treatment—0.02 per cent SnF_2 at pH 3.5 and exposed to lactic acid (Fig. 11).

Low concentrations of stannous fluoride were also effective in protecting dental enamel against 0.1 N lactic acid, pH 5.0 (5-minute exposure). The 5-minute treatment with 0.02 per cent SnF_2 at pH 3.5 caused no visible change in the surface.



Figs. 12-17.—*For legends see opposite page.*

structure. The crystals were shown to be calcium fluoride by electron diffraction. At pH 5.0 (Fig. 13), the sodium fluoride left a layer of larger crystals which appeared definitely cubic. The CaF_2 crystals resembled those identified by previous workers[8, 9, 10] who used long treatments with 2 per cent sodium fluoride solutions at pH 7. In the present work, CaF_2 formed rapidly because, at lower pH values, calcium was liberated more quickly from the tooth structure for reaction with the fluoride.



Figs. 18-19.—Treatment—$SnCl_2$, pH 3 (Fig. 18). Treatment $SnCl_2$, pH 3 and exposed to lactic acid (Fig. 19). Nonfluoride stannous salts were about as effective as stannous fluoride in protecting enamel against the 5-minute acid attack while producing no apparent change in the enamel surface during the 5-minute treatment. This is illustrated by the electron micrographs of enamel treated with 0.576 per cent $SnCl_2 \cdot 2H_2O$ for 5 minutes at pH 3.0, with and without a subsequent exposure to 0.1 N lactic acid pH 5.0, buffer for 5 minutes.

Treating enamel at pH 7.0 with only 0.2 per cent sodium fluoride caused no visible change in the enamel (Fig. 14) during the short treatment times used in these experiments. No electron diffraction studies were made on these samples. Scott, Picard, and Wyckoff[9] have found that neutral sodium fluoride treatments of short duration will leave calcium fluoride on the enamel which is detectable by electron diffraction though not visible by electron microscopy.

Enamel samples treated with sodium fluoride as just described were exposed to the pH 5 lactic acid buffer to determine the effect of acid. After treatments at pH 3.0 and 5.0 with sodium fluoride solutions, the lactic acid

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Figs. 12-17.—NaF treatment at: pH 3 (Fig. 12); pH 5 (Fig. 13); and pH 7 (Fig. 14). Treating polished dental enamel for 10 minutes with 0.2 per cent sodium fluoride at the lower pH values left calcium fluoride crystals on the enamel surface. At pH 7, no change was detected in the enamel.

Lactic acid exposure after NaF treatment at: pH 3 (Fig. 15); pH 5 (Fig. 16); and pH 7 (Fig. 17). The calcium fluoride crystals formed on enamel during the 10-minute treatment with 0.2 per cent sodium fluoride were partially dissolved by the 5-minute exposure to lactic acid at pH 5.0. The neutral sodium fluoride solution imparted no detectable resistance to acid attack on the enamel.

dissolved some of the calcium fluoride. The electron micrographs (Figs. 15 and 16) showed that the crystals were slightly altered; the corners and edges of crystals were less sharp. Because of the obscuring effect of the calcium fluoride coating, nothing could be said about changes in the underlying enamel which might have taken place during the lactic acid buffer exposure.

No significant difference could be found between the effect of the acid buffer on untreated enamel and enamel treated at pH 7 with sodium fluoride (Fig. 17).

Enamel Treated with Nonfluoride Stannous Salts.—In an effort to gain a better understanding of the specific effects of treatments with stannous and fluoride ions in combinations and separately, enamel was treated with several nonfluoride stannous salts. Stannous perchlorate, sulfate, and chloride have been used in this work. These salts hydrolyze much more readily in aqueous solution than does stannous fluoride, and therefore they were studied only at pH 3.0. Even at this pH, hydrolytic products of these salts had a tendency to precipitate, but the solutions could still be utilized for the short times involved. None of the salt solutions produced a visible change in the enamel surface, and all of them protected the enamel surface about as well as did the stannous fluoride. This is illustrated by the electron micrographs of replicas of an enamel surface treated with stannous chloride (0.576% $SnCl_2 \cdot 2H_2O$ = 3 030 ppm Sn^{++}) and of a similarly treated surface followed by exposure to lactic acid buffer (Figs. 18 and 19).

DISCUSSION

It is quite evident from this study that sodium and stannous flouride solutions affect intact enamel surfaces in strikingly different ways. With stannous fluoride solutions no visible change in the treated surface occurs, while the sodium fluoride solutions react with the enamel to deposit calcium fluoride crystals on the surface, the rate of deposition increasing at lower pH values.

There is one aspect of high pH treatments with stannous fluoride solutions that should be pointed out. Aqueous solutions of stannous fluoride are stable only at acid pH values. Above pH 6, stannous ions will precipitate completely as the oxide, leaving essentially a sodium fluoride solution.

$$SnF_2 + 2NaOH + (n - 1)\ H_2O \rightarrow SnO \cdot nH_2O \downarrow + 2NaF \rightarrow SnO \downarrow + 2NaF + nH_2O$$

The first precipitate is a hydrated stannous oxide which will dissolve readily in dilute acid. However, this precipitate will irreversibly dehydrate to form a very insoluble stannous oxide which dissolves only in concentrated acid. These reactions, however, are not instantaneous and require some time to go to completion. Thus, with a fresh solution at pH 7, there was apparently sufficient stannous fluoride still left to react with enamel and have the effect shown. A similar solution aged for several weeks was no longer effective in this manner and acted like a sodium fluoride solution.

The electron micrographs also show the remarkable resistance that surfaces treated with stannous fluoride have to acid etching. The results of the

acid etching of the sodium fluoride-treated surfaces are less definitive, especially in the case of those tests made after treatment at low pH, where the surface structure is obscured by CaF_2 deposits.

The effect of the nonfluoride stannous salt solutions on enamel surfaces appears to be such that the surfaces are made more resistant to acid attack. However, much more study is still required in order to understand and evaluate the combined interaction of stannous and fluoride ions with dental enamel.

All of these observations are consistent with previous reports in the literature on whether or not solutions of these materials dissolved powdered enamel,[7] and with reports on the resulting resistance of the treated enamel to acid attack.[6, 7]

SUMMARY

Electron micrographs of replicas of human enamel surfaces showed that stannous fluoride solutions produced no visible structural alteration, regardless of pH in the range 3 to 7, and of concentrations between 0.02 and 0.4 weight per cent (50-1,000 ppm fluoride).

These treated enamel surfaces showed no appreciable changes when subjected to acid buffer attack at pH 5 for 5 minutes, while an untreated surface showed extensive etching under the same conditions.

Enamel surfaces treated with sodium fluoride showed the typical formation of calcium fluoride crystals on the surface. As the pH of the solution decreased, this deposit formed more rapidly. The effects of an acid buffer on most of these surfaces were indeterminate because of the overlying layer of calcium fluoride. At pH 7, however, where the treatment times used were too short to yield a visible calcium fluoride layer, the results showed that no appreciable resistance to acid etching was imparted.

Nonfluoride stannous salts protected the enamel surface against the acid buffer action in a way which was qualitatively indistinguishable from the action of stannous fluoride, using the methods and technics described in this report.

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THE EFFECT OF PILOCARPINE ON THE SUBMAXILLARY GLAND OF THE RAT

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EXPERIMENTS performed with rats and hamsters[1] indicate that a reduction in salivary flow, produced by gland extirpation or duct ligation, results in a significant increase in experimental dental caries. Preliminary data from our laboratory indicate that an increased salivary flow produced by pilocarpine administration may be associated with a significant reduction in caries experience under certain experimental conditions.[2] However, because this drug has undesirable side effects, it was felt that the effect of its administration on the histologic appearance of the salivary glands should be investigated. Hence, the purpose of this paper is to report the effects of pilocarpine upon the histology of the submaxillary gland of the rat after both limited and prolonged drug administration.

EXPERIMENTAL

Adult male albino rats (Sprague-Dawley strain) were used in 2 experimental series. Series I was composed of 32 animals (350 to 400 gram body weight) of which 24 were "stomach-tubed" with a single dose of 12 mg. of pilocarpine. Four animals were sacrificed at each of the experimental periods of 30 minutes, 1, 2, 5, 12, and 24 hours following pilocarpine administration. The remaining 8 animals served as controls.

Series II was composed of approximately 100 weanling male animals which were divided into 5 groups. Group 1 received a cariogenic diet containing 10 mg. of desiccated thyroid powder per gram of diet. Groups 2, 3 and 4 were "stomach-tubed" daily with 2, 6, and 12 mg. of pilocarpine hydrochloride. Group 5 served as controls. Three animals from each group were sacrificed at 1, 2, 4, 6, 8 and 12 weeks following the beginning of the pilocarpine administration.

Submaxillary glands from both series were removed and fixed in Bouin's solution, embedded in paraffin and sectioned at 8 microns. Sections from each of the animals were stained in the following manner: (1) hematoxylin and eosin; (2) periodic acid-Schiff (PAS) using orange G and celestin blue as counterstains[3]; (3) methyl-green pyronin for ribonucleic acid (RNA) determination.[4] With this latter stain, sections which had been previously incubated in a

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Received for publication Nov. 15, 1957; revised by authors March 20, 1958.

sterile solution of crystalline ribonuclease (0.25 mg./ml.) for 2 hours at 37° C. were used as the control sections. Any material which stained after the digestion was not considered to be RNA.

RESULTS AND DISCUSSION

The adult rat submaxillary gland is composed of 4 principal portions: interlobular ducts, intralobular ducts, granular tubules, and acini (Fig. 1). The interlobular duct is composed of an epithelium which normally varies from

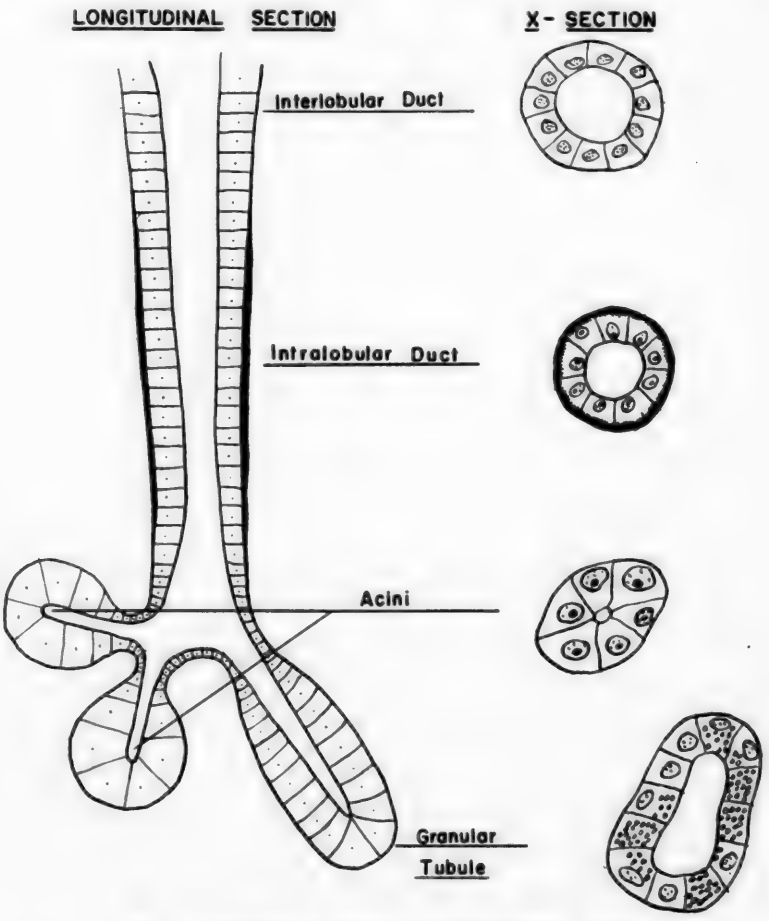

Fig. 1.—Anatomy of normal rat submaxillary gland.

cuboidal to low columnar in type. Characteristically, the nuclei are located next to the lumen of the duct and occasionally in a more basal position. These cells have no apparent secretory function or secretory granules, and are relatively constant in appearance.

The intralobular duct portion of the gland is composed of low columnar cells lining a well-defined lumen. The nuclei are spherical, centrally oriented

in the cells, and have a single prominent nucleolus. Frequently, the cytoplasm of these cells will show basal striation. Emptying into the intralobular ducts is the tubular portion of the gland. These tubules have been the stimulus for much research because of their apparent interrelationships with the thyroid and sex hormones as well as experimental caries incidence.[5] This distal portion, which in the past has been referred to as the "granular tubule," is outstanding for the presence in its cells of numerous, acidophilic, highly refractile granules. These granules occur in the tubular cells of rats and mice but not other rodents. The cells themselves have an abundant cytoplasm which is weakly PAS positive. Their nuclei are spherical with dispersed chromatin, and 1 or 2 nucleoli. In a cell which contains no granules the nuclei are centrally positioned, whereas, a cell filled with granules has a nucleus away from the duct lumen or basal in position. No RNA is found histochemically in this portion of the gland. Although these granular tubules frequently empty into the duct system, the relationship between the tubules and acini is less apparent. These tubules may be separate and distinct branches of the duct system, or they may be in an intermediate anatomic relationship between the acini and the duct system.

The acini compose the major portion of a young animal's gland and somewhat less than one-half that of an adult rat's, the granular tubules making up the difference. Each acinus is composed of several pyramidal-shaped cells which have a somewhat centrally placed nucleus showing a single prominent nucleolus. The cell cytoplasm is strongly PAS positive and has its RNA content concentrated in the basal portion of the cell. Even though these staining reactions are somewhat typical of a mucus-secreting cell, the morphology of the cells and the appearance of their nuclei are more like those of serous-secreting cells. The true secretory product of these cells remains to be identified.

TABLE I

THE EFFECT OF PILOCARPINE ADMINISTRATION UPON SUBMAXILLARY GLAND HISTOLOGY AND RNA CONTENT

	TUBULAR PORTION OF INTRALOBULAR DUCT		SEROUS ACINI	
	CELLS CONTAINING GRANULES	PAS + MATERIAL IN DUCTS	PAS REACTION	RNA†
30 min.*	Normal	+	Normal and diffuse	B – 2
1 hr.	Marked decrease	+++	Intense	B – 2
2 hr.	Normal	++++	Intense, cytoplasm vacuolated	BD – 3
5 hr.	Normal	++	Normal	BD – 4
12 hr.	Normal	+	Normal	BD – 2
24 hr.	Normal	+	Normal	B – 2

*Time after administration.
†B = basal; D = diffuse in cytoplasm; 1-4 = increasing intensity of stain.

Thirty minutes following the administration of 12 mg. of pilocarpine by a stomach tube, no histologic alterations were noted (Table I). After 1 hour there was a marked decrease in the number of tubular cells containing granules. Even more apparent was the stronger PAS reaction in the acinar cells. At this time a considerable number of the ducts were filled with a PAS-positive material. From 2 to 24 hours the principal changes were in the acinar PAS

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reaction (strongest at 2 hours), and the acinar RNA content which gradually increased to a maximum at 5 hours and was within normal limits at 12 hours. No nuclear changes were observed.

These results suggest that pilocarpine has its most pronounced effect upon the acinar portion of the gland, and that the apparent height of cellular activity occurs at 2 to 5 hours after pilocarpine administration. However, profuse salivation was evident in these animals 30 to 45 minutes after pilocarpine was given, and this flow persisted for about 30 minutes.

Although some alterations in histologic appearance were observed in the series which was designed to study the effects of daily pilocarpine administration over extended periods of time, no apparent pathosis was noted. The control glands showed a progressive increase in acinar cell RNA intensity with a maximum at about 6 weeks (animal age approximately 72 days). The animals receiving desiccated thyroid in the diet seemed to have a greater amount of RNA at each period as compared to the controls. Pilocarpine administration, at all 3 levels, had little effect on acinar RNA. About the sixth week, there was a definite increase in acinar RNA content, although the total amount appeared little different from the controls. However, it was observed that RNA in these 3 pilocarpine-treated groups was invariably spread diffusely throughout the cell cytoplasm and not confined to a basal or perinuclear position as in the thyroid-fed and control groups (Table II).

TABLE II
EFFECT OF PILOCARPINE, GIVEN DAILY, UPON SUBMAXILLARY GLAND RNA

	1 WEEK	2 WEEKS	4 WEEKS	6 WEEKS	8 WEEKS	12 WEEKS
Desiccated thyroid	2 B	3 BD	3 B	3 B	4 B	3 B
2 mg. pilocarpine	1 D	2 D	1 D	2 BD	3 D	3 D
6 mg. pilocarpine	1 D	2 D	1 D	2 BD	4 BD	2 D
12 mg. pilocarpine	1 D	2 D	1 D	4 BD	2 D	1 D
Control	1 B	2 B	2 B	3 B	2 B	2 B

Location of RNA: B = basal; D = diffuse in cytoplasm.
Intensity of stain: 1—least; 4—greatest.

Submaxillary glands from the control and desiccated thyroid groups both exhibited a progressive increase with age in the size of the granular tubules as well as in the number of cells containing granules, the thyroid-treated group showing a somewhat greater increase. In contrast to the effect of desiccated thyroid, pilocarpine had no apparent effect on the tubular portion of these glands, even after 12 weeks of daily administration.

In a previous experiment, 4 groups of animals which were on an experimental regimen identical to that used here (3 levels of pilocarpine given daily, and desiccated thyroid administered in the diet) were examined for dental caries experience. Pilocarpine in this earlier study was found to have a distinct anticariogenic effect. It appears then that this drug may be a useful experimental tool in studying dental caries in the rat since the results of the present study also show that no apparent pathosis exists in the submaxillary gland even after prolonged drug administration.

Although no salivary flow measurements were made on these animals, by gross observation it was apparent that a profuse flow resulted from the administration of pilocarpine at every level even after 12 weeks of daily therapy.

SUMMARY

Pilocarpine, when given to adult male rats, had its most pronounced effect upon the acinar portion of the submaxillary salivary gland 2 to 5 hours after administration. This effect was an increased intensity of the periodic acid-Schiff reaction and a spreading out of the methyl-green pyronin (RNA) reaction in the cell cytoplasm. Prolonged administration of pilocarpine (daily for 12 weeks) produced no apparent pathosis in the submaxillary gland, although gross observations revealed that the drug regularly stimulated a profuse and voluminous flow throughout the entire experimental period.

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THE INHIBITORY ACTION OF SALIVA ON THE IN VITRO GROWTH OF BETA HEMOLYTIC STREPTOCOCCI

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THE incidence of beta hemolytic streptococcal infections in the oral cavity has been reviewed and discussed.[1] Although this microorganism possesses both invasive and toxigenic characteristics, the number of local and systemic infections following endodontic treatment, periodontal therapy, tooth extraction, or other oral surgical procedures are relatively few. Hemolytic streptococci are seeded to the mouth from the oro- and nasopharynx and the external environment but rarely become members of the resident oral microbiota. The failure of certain species of microorganisms to establish themselves as members of the indigenous flora is associated with such regulating factors as the nature and type of diet, the masticatory process itself, microbial metabolic products and the flushing, chemical composition and the antibacterial nature of saliva.

Dold and Weigmann[2] demonstrated that human saliva inhibited the in vitro growth of the diphtheria bacillus. Bibby, Clough, and Hine,[3] employing the cup-well method, found that saliva inhibited the growth of 110 of 169 species, mostly air- and water-borne microorganisms. They observed only a very slight antibacterial activity of saliva toward the *Streptococcus hemolyticus.*

Because lysozyme[4] has a broad range of activity against many saprophytic microorganisms, many investigators have assumed that the antibacterial activity of any of the body fluids known to dissolve micrococci is due to this enzyme. Thompson[5] confirmed the presence of lysozyme in saliva and provided in vitro experimental evidence for the presence of other antibacterial factors.

Van Kesteren, Bibby, and Barry[6] substantiated Thompson's results and concluded that several antibacterial factors are present in saliva, one of which is definitely lysozyme. Thompson[7, 8] and Bethege, Soehring, and Tschesche,[9] and Hegemann[10] showed that the in vitro inhibitory action of saliva toward the diphtheria bacillus was due to hydrogen peroxide production by the oral alpha streptococci. Berger[10, 11] corroborated the biological production of hydrogen peroxide by oral alpha streptococci and its role in inhibiting the in vitro growth of the diphtheria bacillus. Rosebury, Gale, and Taylor[12] observed that streptococci could be separated into several systems, dependent on their mode of inhibitory activity: those which produce a complete and diffusible inhibition on media not containing blood or potato juice (catalase

This study was supported in part by a grant-in-aid D-375 from the National Institute for Dental Research of the National Institutes of Health and the Henry Spenadel Research Fund.

Read at the 36th Annual Meeting of the International Association for Dental Research, Detroit, Michigan, March, 1958.

Received for publication Nov. 21, 1957; revised by authors April 30, 1958.

containing materials) due probably to the in vitro production of hydrogen peroxide, and those which produce their inhibitory effect from some other antibiotic material. Nickerson, Kraus, and Perry[13] found that peroxidase in normal saliva is predominantly of glandular origin with a smaller amount of activity contributed by leukocytes; none was demonstrated in the salivary streptococci. They found catalase to be absent from glandular secretions and presumably of bacterial origin. Kraus, Nickerson, Perry and Walker[14] concluded that the majority of aerobic bacteria in human saliva form hydrogen peroxide in vitro, and that absence of peroxide in saliva under in vivo conditions is explained by the presence of catalase and peroxidase.

It was the purpose of this investigation to study the factors which limit the establishment of beta hemolytic streptococci as members of the oral flora.

Fig. 1.—Upper half of plate contains 25 per cent heated saliva; lower half contains 25 per cent fresh saliva in brain heart infusion agar. Streaks of test microorganisms from left to right: 1—*M. lysodeikticus*. 2, 3, 5, 6—Beta hemolytic streptococcal strains. 4—*M. pyogenes* var. *aureus*. Note that only *M. pyogenes* var. *aureus* grew on the portion containing fresh saliva.

EXPERIMENTAL METHOD AND RESULTS

Determination of the Presence of an "anti-Hemolytic Streptococcus" Factor in Saliva.—Brain heart infusion agar (Difco) with the addition of sufficient agar to give a 2 per cent concentration was used as the basal medium throughout this study. Stimulated saliva was collected by chewing baseplate wax (Alcizon, Myco) previously tested and shown not to be bactericidal, and added in 2.5 ml. aliquots to give a 25 per cent concentration by volume to 10 ml. of the basal medium cooled to 42° C. Plates were poured, and the agar surfaces streaked in parallel rows with loopfuls of freshly prepared 0.85 per cent saline suspensions (of a density comparable to a No. 10 McFarland $BaSO_4$ standard) of the following microorganisms: *Micrococcus lysodeikticus*, various strains of group A beta hemolytic streptococci, *Micrococcus pyogenes* var. *aureus*, *Neisseria*

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flavescens, and *Bacillus cereus.* The plates were incubated at 37° C., and observed after 24 and 48 hours. Suitable controls were utilized consisting of the medium without saliva, and the medium with 25 per cent heated saliva (100° C., 15 minutes).

The basal medium supported luxuriant growth of many of the salivary microorganisms, the majority of which appeared as colonies in the medium. No surface growth of the beta hemolytic streptococcal strains or *M. lysodeikticus* occurred. *M. aureus, N. flavescens,* and the *B. cereus* showed sparse to moderate growth. The heated saliva supported the growth of the hemolytic streptococci and other test organisms equally as well as, or slightly better than, the basal medium without saliva. No colonies of oral microorganisms developed in the heated saliva plate.

The Effects of Bacteria-Free Saliva Filtrates on Hemolytic Streptococci.—Stimulated saliva was collected as previously noted from 4 individuals at different times and filtered through a Seitz S 1 filter. The clear filtrate was divided into two aliquots, one of which was heated at 100° C. for 15 minutes to destroy any soluble heat-labile factors. Both portions were tested as previously described.

Neither the heated nor unheated filtrates appeared to have any effect on the surface growth of the test organism. No colonies of the oral microbial flora developed in the depths of the medium.

The Concentration of "anti-Hemolytic Streptococcus" Factor in the Salivas From Different Individuals.—Over 200 specimens of stimulated saliva were obtained from 16 apparently normal individuals (13 males, 3 females) on various days and tested as previously described.

The *anti*-hemolytic streptococcus factor was found in the saliva specimens of all individuals tested. The growth of *M. lysodeikticus* was always inhibited while *M. aureus, N. flavescens,* and *B. cereus* growth varied from poor to good but was never as luxuriant as the growth on either of the control plates. The antibacterial activity of the saliva toward the latter three mircoorganisms varied with specimens from various individuals and also with saliva samples collected from the same individual on different days. Inhibition of *M. lysodeikticus* and the hemolytic streptococcal strains showed little variation.

Differences in Strain Sensitivity of Beta Hemolytic Streptococci to the Salivary Factor.—Seven strains of hemolytic streptococci recently isolated from patients in the Pediatric Ward of Bellevue Hospital, and the Veterans Administration Hospital were tested, along with 5 strains maintained in the culture collection of our laboratory.

Growth of all strains was inhibited on plates of the medium to which fresh stimulated saliva was added. Heated saliva had no *anti*-hemolytic streptococcus effect.

The Effect of the Addition of Oral Bacteria to Heated Saliva on the Growth of Beta Hemolytic Streptococci.—Heavy inocula of oral alpha streptococci or the mixed surface growth obtained from an aerobic blood plate previously streaked with a loopful of saliva were added to heated saliva basal medium. The surface was streaked with the test microorganisms.

Test and control plates (containing unheated saliva) showed the same degree of inhibition of the test organisms.

The Effect of Egg White Lysozyme on the Growth of Hemolytic Streptococci.—Fresh egg white was prepared and serial saline dilutions were added to 10 ml. volumes of brain heart infusion agar. Plates were poured and the agar on solidifying was streaked with the test organisms.

The egg white lysozyme inhibited growth of *M. lysodeikticus* in a 10^{-5} dilution. No inhibition of the hemolytic strains or the other organisms occurred in a dilution of 10^{-1}.

The Effect of Tear Lysozyme on the Growth of Beta Hemolytic Streptococci.—Tears were collected from several individuals and several drops (approximately 0.1 ml.) were added to 5 ml. of physiological salt solution to give a 1:50 concentration. Serial dilutions to 1:5000 were then made from this volume and added to the basal medium.

Tears in dilutions of 1:1000 inhibited the growth of *M. lysodeikticus* but showed no inhibitory action on the various strains of beta streptococci or other test organisms.

The Effect of Purified Lysozyme on the Growth of Hemolytic Streptococci.—Ten milligrams of purified powdered lysozyme (Mann Research Corp.) were added to 10 ml. phosphate buffer (pH 6.2). It was tested in serial dilutions of 10^{-3} to 10^{-6}.

M. lysodeikticus was inhibited in a 10^{-5} dilution. A 10^{-3} dilution had no effect on the strains of hemolytic streptococci or the other test organisms.

The Effect of Variations in pH and Temperature on the "anti-Hemolytic Streptococcus" Factor.—Saliva specimens were adjusted to pH 4.5 to 5.0, heated in water bath at 100° C. for 5 minutes, and adjusted to pH 6.6 to 7.0 with N/1 NaOH. Thompson[5] has reported that such a procedure will not destroy salivary lysozyme but will inactivate the other antibacterial factors. The saliva specimens so treated were added to the basal medium in a 25 per cent concentration and the test organisms streaked on the surface.

No growth of *M. lysodeikticus* occurred. The hemolytic streptococcal strains and the other test organisms grew unrestricted.

In Vitro Hydrogen Peroxide Production by the Oral Microbiota of Stimulated Saliva.—Benzidine potato juice basal medium plates were prepared by adding 60 mg. of benzidine and 2 ml. of freshly Seitz filtered potato juice to 100 ml. of medium.[8] Twenty-five per cent of unheated saliva was added to the above medium and plates were poured. The surface was streaked with the test organisms.

Numerous colonies in the depths of the agar were surrounded by brownish zones indicative of hydrogen peroxide production. On the surface of the medium no growth of *M. lysodeikticus* or the hemolytic streptococcal strains was noted. *M. aureus* and *B. cereus* grew unrestricted. The addition of 2 drops of 3 per cent hydrogen peroxide to the surface growth of *M. aureus* or *B. cereus* produced violent bubbling indicating the presence of catalase.

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The Effect of Various Concentrations of Hydrogen Peroxide on the Growth of Hemolytic Streptococci.—Hydrogen peroxide (3 per cent) was added to 10 ml. amounts of the basal medium to give final concentrations of 0.3, 0.1, 0.05, 0.03, and 0.01 per cent, respectively. Heated saliva in 2.5 ml. aliquots was added to each tube of medium. Plates were poured and the surface streaked with the test microorganisms: *M. lysodeikticus, M. pyogenes aureus,* and 4 strains of beta hemolytic streptococci. Fresh saliva medium controls were utilized.

Growth of all test organisms occurred on the 0.01 per cent hydrogen peroxide heated saliva plates. Two strains of hemolytic streptococci were inhibited in 0.03 per cent concentration while the other 2 strains grew sparsely. Growth of *M. lysodeikticus* and *M. aureus* was not affected. The 0.05 per cent concentration prevented growth of the 4 hemolytic streptococcal strains. No effect on the other 2 test organisms was noted. The 0.1 per cent concentration permitted slight growth of *M. lysodeikticus* and *M. aureus.* At 0.3 per cent hydrogen peroxide concentration, the growth of all the test organisms was inhibited. The results obtained on the 0.03 per cent and the 0.05 per cent hydrogen peroxide simulated the growth on the 25 per cent fresh saliva plates with the exception of *M. lysodeikticus* which grew on the former plates.

The Effect of the Addition of Laked Blood, Potato Juice, or Catalase to the Basal Medium.—Twenty-five per cent saliva basal medium plates containing 2 per cent of a 1:10 laked blood or 2 per cent freshly prepared sterile potato juice were poured. Additional plates containing 10 units of catalase per milliliter of medium were also prepared. A basal medium without saliva but containing either laked blood, potato juice, or catalase was used as control.

(1) Growth of all the hemolytic strains was inhibited on the 25 per cent unheated saliva basal medium containing laked blood, potato juice, or catalase. (2) All strains grew well on the control plates. (3) The activity of the catalase in the test plates was confirmed by vigorous bubbling on the addition of 2 drops of 3 per cent hydrogen peroxide.

The Demonstration of "anti-Hemolytic Streptococcus" Factor Under Anaerobic Conditions of Incubation.—Twenty-five per cent saliva basal medium plates and basal medium plates without saliva were poured. The surface was streaked with *M. lysodeikticus, C. diphtheriae* var. *gravis* and *mitis, M. aureus,* and 3 strains of beta hemolytic streptococci. All plates were incubated in a Brewer jar for 24 hours at 37° C.

The control plates showed good growth of all test microorganisms. On the 25 per cent saliva plates, no growth of *M. lysodeikticus* and the strains of beta hemolytic streptococci occurred. Growth of *M. aureus* and *C. diphtheriae* varieties was not restricted.

DISCUSSION

Freshly stimulated whole saliva contains a factor which under in vitro experimental conditions inhibits the growth of beta hemolytic streptococci. This factor is effective against both recent streptococcal isolates and stock culture strains and has been found in over 200 specimens of saliva collected

on various days from 16 apparently normal individuals. It appears to be distinct from lysozyme since Thompson's[5] method for destroying antibacterial factors other than lysozyme negates its action. Furthermore, egg white, tear and purified crystalline lysozyme in low dilution have no observable effect on the strains of beta hemolytic streptococci utilized in this study.

The sediment of centrifuged saliva washed 3 times with physiological saline to remove any soluble fractions and then suspended in basal medium proved as effective as the whole saliva in inhibiting the growth of beta hemolytic streptococci. Heated saliva never produced inhibition; in fact, in some instances the addition of the heated saliva improved the growth of the test organisms. Bibby[15] stated that filtered saliva kills hemolytic streptococci. We were unable to confirm this finding in as much as our Seitz or Berkfeld filtered samples had no apparent inhibitory effects.

The addition to heated saliva basal medium plates, of heavy inocula of oral alpha streptococci or the mixed surface growth obtained from an aerobic blood plate previously streaked with a loopful of saliva, inhibited the growth of the beta hemolytic streptococcal strains. It appears that the *anti*-hemolytic streptococcus factor is associated with the viable oral microorganisms.

Bethege,[9] Thompson,[8] Berger[10] and others have associated the inhibitory action of saliva on the diphtheria bacillus with the in vitro hydrogen peroxide production by alpha streptococci. We have noted the in vitro production of hydrogen peroxide by oral microorganisms and have observed that the addition of 0.03 to 0.05 per cent hydrogen peroxide to brain heart infusion agar (2 per cent) will inhibit the surface growth of the beta hemolytic streptococci. In the presence of laked blood, potato juice, or lyophilized catalase, hydrogen peroxide is rapidly decomposed and its antibacterial activity is negated. This is in accord with the observation of Su,[16] Annear,[17] Sherwood,[18] Hegemann,[19] Berger,[11] and Rosebury,[12] and their co-workers. The *anti*-hemolytic streptococcus factor appears to retain its activity in the presence of catalase or materials containing catalase. We believe that the in vitro production of hydrogen peroxide is a coincidental phenomenon and its activity is distinct from that of the *anti*-hemolytic streptococcus factor. Further supporting evidence that this factor is not hydrogen peroxide, is an ability to manifest activity under anaerobic conditions wherein hydrogen peroxide is presumably not produced.

The presence of catalase and the absence of hydrogen peroxide in freshly stimulated saliva is confirmed. This is in agreement with the findings of Appleton,[20] Berger,[10] Burnett and Scherp,[21] and Kraus, Nickerson, Perry, and Walker.[14] Since 1 molecule of catalase can decompose 2,600,000 molecules of hydrogen peroxide per minute, a rate of action much faster than for most enzymes,[22] it is doubtful whether the in vivo production of hydrogen peroxide is of significance. It is likely that any antibacterial effect which limits the establishment of beta hemolytic streptococci as residents of the oral cavity is of a more specific nature.

The question as to whether the inhibition of the in vitro growth of the beta hemolytic streptococcal strains is due to some antibiotic factor produced

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during the metabolism of the oral microbiota or whether an essential factor for growth of these strains is consumed during the growth of oral microorganisms is at this time not resolvable.

SUMMARY

Freshly stimulated saliva exhibits an inhibitory action toward the hemolytic streptococci under the stated in vitro experimental conditions. The factor is distinct from lysozyme, not filterable through the Seitz sterilizing filter; it may be associated with the oral microbial flora, and is effective in inhibiting growth of both stock and recent beta hemolytic streptococcal isolates. Since the factor inhibits the growth of hemolytic streptococci in the presence of laked blood, potato juice, or lyophilized catalase and is active under anaerobic conditions, we conclude it is distinct from hydrogen peroxide production in vitro.

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THE MICROHARDNESS OF ENAMEL AND DENTIN

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THE hardness of enamel and dentin has been determined by a variety of methods including abrasion,[1, 2] pendulum,[3] scratch,[4-7] and indentation[8-13] technics. Since the hardness of enamel and dentin has been shown to have considerable local variations, the methods using a microscratch or microindentation have been preferred. One of the more common types is the Knoop diamond indenter[14] which has been used by a number of investigators.[10, 12, 15, 16] It should be mentioned, however, that in spite of the fact that the indentations are extremely small, they still represent a macroindentation when compared to the microstructure of enamel and dentin.

The majority of the published hardness data for enamel and dentin has been measured on ground sections, although several papers[10, 13] reported the hardness of intact enamel surfaces. The conclusions in regard to the difference in hardness from one section of a tooth to another are at times in variance with each other. This study of dentin and enamel was undertaken in an attempt to establish any trends in hardness existing from one area of a tooth to another or between different types of teeth. With this purpose in mind, this research did not attempt to relate the hardness values to the histologic tooth structure, but a sufficiently large number of hardness measurements were made so that the data could be treated on a statistical basis.

EXPERIMENTAL

Specimen Preparation.—Mature, freshly extracted, noncarious teeth were imbedded in Ward's Bio-Plastic by suspending them in a Vaughn ring containing the polymer mixed with the catalyst and accelerator. The incorporated air was removed by degassing in a vacuum chamber, after which the polymerization was hastened by placing the ring in an oven at 50° C. for 4 hours or more.

The imbedded teeth were sectioned by using a water-spray cooled carborundum wheel, 0.33 mm. thick. The specimens were cut so that the sections were 1 to 2 mm. thick, the first cut being made at or slightly below the occlusal surface or incisal edge. Specimens cut in a direction mesial to distal or buccal to lingual were cut so that the first section was the lingual or mesial surface, respectively. The thickness of each section was measured with a micrometer and the surfaces to be tested were polished by the successive use of 240A, 400A, and 600A Norton Tufback Speed wet paper supported by a glass slab, followed by

This report represents the partial results of studies supported by Contract No. D-462 between the U. S. Public Health Service, National Institute of Health, and the University of Michigan.

Received for publication Dec. 9, 1957.

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TABLE I. TYPICAL HARDNESS MEASUREMENTS ON TOOTH SECTIONS

TOOTH TYPE	SECTIONED	POSITION OF SECTION BELOW CUSP TIP (MM.)	LOCATION OF INDENTATIONS	KNOOP HARDNESS NUMBERS			
				ENAMEL		DENTIN	
				KHN ± S.D.	NO. OF INDENTATIONS	KHN ± S.D.	NO. OF INDENTATIONS
Lft. max. 2nd molar	Parallel to occlusal	2.02	2.35 mm. from buccal	371 ± 35	52	77 ± 13	5
		4.71	1.69 mm. from mesial	390 ± 32	21	78 ± 3	32
		5.40	1.78 mm. from lingual	368 ± 32	23	67 ± 4	51
		8.04	1.71 mm. from distal	----	--	68 ± 4	29
Lft. mand. 1st premolar	Parallel to occlusal	0.51	1.44 mm. from buccal	364 ± 43	28	66 ± 11	6
		2.77	2.96 mm. from buccal	292 ± 28	17	62 ± 6	17
		5.23	2.57 mm. from buccal	309 ± 45	9	71 ± 5	19
		5.92	1.43 mm. from buccal	308 ± 47	6	65 ± 4	15
Rt. mand. 1st premolar	Parallel to occlusal	0.78	2.14 mm. from buccal	344 ± 49	22	60 ± 6	8
		2.92	2.14 mm. from buccal	327 ± 20	15	72 ± 6	26
		4.98	1.14 mm. from buccal	345 ± 21	9	61 ± 5	25
		5.67	0.91 mm. from buccal	335 ± 25	6	64 ± 5	18

separate polishings with Shamva and CRO metallographic polishes at low speeds on a wet polishing wheel. This polishing procedure removed approximately 0.18 mm. of a tooth section.

The distance of the surface to be tested from the reference surface could be estimated knowing the thickness of the sections, and the amounts removed by the carborundum wheel and the polishing procedure.

The polished specimens were mounted on glass microscope slides by attaching the edges of the specimens to the glass with red utility wax, and using a hand press to ensure that the surface to be tested was parallel to the table of the hardness tester.

Hardness Testing.—An "MO" Tukon microhardness tester was used with a Knoop diamond indenter to determine the hardness values. The polished sections mounted on microscope slides were fastened to the mechanical specimen stage of the hardness tester. Loads from 25 to 200 Gm. on the indenter were investigated and it was found that a 50-gram load applied to the specimen for 15 seconds represented optimum conditions. This procedure resulted in well-defined indentations with a minimum of fractures around the edges. The average length of the long diagonals for enamel and dentin was 0.046 and 0.101 mm., respectively. The ratio of the long to short diagonal of the indentations, which should be approximately 7.1, was not taken as a criterion for a satisfactory indentation since contraction of the short diagonal occurred due to retarded elastic recovery. This phenomenon was particularly pronounced in dentin. The main criteria for accepting an indentation value were clearness of outline and absence of flaws in the tooth in the area of the measurement.

The indentations were made in a line from mesial to distal or from buccal or labial to lingual for sections cut parallel to the occlusal surface or incisal edge. For sections cut mesial to distal or buccal to lingual the measurements were made in the direction of the distal or lingual surface. In one case (tooth No. 25) the indentations were taken in a transverse section around the tooth at a definite distance from the enamel edge or the dentinoenamel junction.

RESULTS

Typical experimental results obtained on transverse sections of teeth are tabulated in Table I. Three types of teeth are listed along with the type and position of the sections, the location of the indentations, the number of indentations, the average Knoop hardness numbers for each section, and the respective standard deviations.

The Knoop values for enamel varied considerably from one location to another in the same section as indicated in Table I by the standard deviation values ranging from ±20 to ±49. Pronounced differences existed between the hardness values of different sections of the same tooth. The average hardness values of enamel for the 3 teeth listed were 376, 326, and 338, respectively. Thus, smaller deviations were observed between these averages for different teeth than between average values for different sections of a single tooth.

In general, no definite trend in the hardness of enamel was observed from the occlusal surface to the cervical margin or from the dentinoenamel junction to the outer enamel surface. Some slight indications were found that the enamel was harder at the cusp and outer surface than at the cervical margin or the dentinoenamel junction, but the order of the differences was approximately 25 Knoop numbers, which is less than the average standard deviation, and thus no definite statement can be made.

It should be pointed out that due to the large number of hardness determinations, single values having a deviation greater than 2 times the standard deviation from the average hardness value were discarded on the basis that they were outside the range of experimental errors and probably represented inordinately high or low areas of hardness. These values possessing large deviations from the average will be discussed later in this section.

The Knoop hardness numbers of the dentin for a single transverse section also varied and standard deviations from ± 3 to ± 13 were found. Different average hardness values were obtained for the sections of the same tooth and no general trend in the hardness of dentin in teeth was observed. The average standard deviation for dentin is only ± 5; however, this represents approximately 7.5 per cent error compared with about 10.8 per cent error for the enamel values.

Although no general trends were observed in the hardness values of dentin and enamel from one portion of a tooth to another, in general more variation in hardness values was obtained in the first section below the occlusal surface or the incisal edge than in the remaining sections.

The hardness values obtained on 5 sections of tooth No. 10 are presented in Fig. 1. The tooth, a left mandibular first premolar, was sectioned parallel to the occlusal surface and the Knoop hardness values were measured on surfaces 1 to 5 in a line from the mesial to the distal edge. Thus, the plot represents the hardness at various distances from the mesial edge. The points have been connected only to show continuity of surface and not to represent the change of hardness between points. The dispersion of the enamel values is very noticeable, while the variation in dentin values is less pronounced. The decrease in the thickness of enamel and the increase in thickness of dentin from surface 1 to 5 is also evident. The hardness values of the dentin and enamel in the region of the dentinoenamel junction were the lowest values obtained. Several reasons are possible for this fact: (1) the indentations in the adjacent enamel region had a greater probability of being partly in a longitudinal soft layer than those farther from the dentinoenamel junction; (2) the indentations in the adjacent dentin may be affected by a high concentration of dentinal fibers and/or interglobular areas; and (3) the polishing of a transverse section of enamel and dentin, having different hardness values, results in somewhat curved surfaces near the dentinoenamel junction which would yield too large an indentation and thus too low a Knoop hardness number.

Various types of teeth were sectioned either transversely or longitudinally and the hardness values of dentin and enamel obtained are reported in Table

II. The Knoop hardness numbers listed are weighted averages of individual values obtained on all sections of a single tooth. The standard deviation and the number of indentations for each tooth are also tabulated. If the number of indentations for each tooth is disregarded, the over-all average hardness value of enamel and dentin is 343 and 68, respectively. The standard deviations for these over-all averages are ±23 and ±3 which are low when compared with the standard deviation for a single tooth and, therefore, no trend could be detected between the hardness values of the various types of teeth.

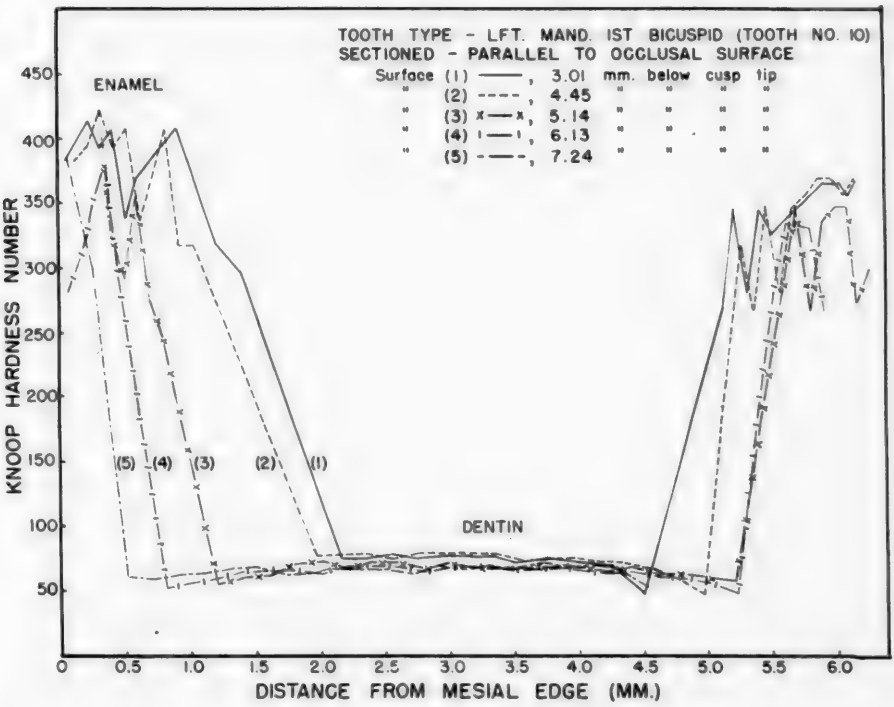

Fig. 1.—Enamel and dentin hardness values of tooth sections.

The enamel hardness value of 418 ±60 for tooth No. 25 and the dentin hardness values of 76 ±8 for tooth No. 26 and 60 ±7 for tooth No. 14 represent values having the largest deviations from the over-all averages. These values were a result of high or low hardness values in a single section of the tooth which only emphasizes that as large or larger variations can be present in a single tooth as between two teeth.

As previously mentioned, hardness values having deviations greater than twice the standard deviation were discarded as being outside the region of experimental error. The number of these discarded values, both high and low, amounted to approximately 15 per cent of the total number of indentations.

TABLE II. SUMMARY OF HARDNESS MEASUREMENTS ON ENAMEL AND DENTIN

TOOTH			ENAMEL			DENTIN		
NUMBER	TYPE	SECTIONED	KHN	S. D.	NO. OF INDENTATIONS	KHN	S.D.	NO. OF INDENTATIONS
1	Rt. mand. 3rd molar	Parallel to occlusal	329	± 40	107	64	± 7	103
2	Lft. max. 3rd molar	Buccal to lingual	349	± 42	84	67	± 4	173
3	Lft. max. 3rd molar	Mesial to distal	332	± 38	180	68	± 7	82
4	Lft. max. 2nd molar	Parallel to occlusal	376	± 33	89	72	± 4	123
5	Lft. max. 2nd molar	Parallel to occlusal	330	± 38	92	69	± 4	130
6	Rt. mand. 1st molar	Parallel to occlusal	346	± 26	52	74	± 5	147
7	Lft. max. 2nd premolar	Parallel to occlusal	374	± 43	175	70	± 4	88
8	Lft. mand. 1st premolar	Parallel to occlusal	326	± 39	60	66	± 5	57
9	Lft. mand. 1st premolar	Parallel to occlusal	314	± 28	61	69	± 5	62
10	Lft. mand. 1st premolar	Parallel to occlusal	329	± 25	59	69	± 4	111
11	Rt. mand. 1st premolar	Parallel to occlusal	338	± 36	52	65	± 6	77
12	Rt. mand. 1st premolar	Parallel to occlusal	331	± 28	69	68	± 3	81
13	Rt. mand. 1st premolar	Parallel to occlusal	332	± 31	90	69	± 3	113
14	Lft. max. 1st premolar	Buccal to lingual	333	± 46	154	60	± 7	75
15	Lft. mand. cuspid	Parallel to incisal	329	± 32	42	68	± 4	112
16	Lft. mand. cuspid	Parallel to incisal	337	± 31	95	71	± 4	87
17	Lft. mand. cuspid	Parallel to incisal	354	± 35	95	64	± 7	98
18	Rt. mand. cuspid	Parallel to incisal	316	± 28	63	67	± 7	107
19	Rt. mand. cuspid	Parallel to incisal	349	± 37	76	70	± 4	87
20	Rt. mand. cuspid	Parallel to incisal	336	± 32	82	66	± 7	81
21	Rt. mand. cuspid	Buccal to lingual	343	± 43	73	72	± 8	49
22	Lft. max. cuspid	Parallel to incisal	358	± 52	107	69	± 6	30
23	Lft. mand. lat. incisor	Parallel to incisal	341	± 30	51	70	± 4	74
24	Rt. mand. lat. incisor	Parallel to incisal	342	± 28	54	68	± 4	83
25	Rt. mand. cent. incisor	Parallel to incisal	418	± 60	89	67	± 4	57
26	Lft. max. cent. incisor	Parallel to incisal	359	± 62	186	76	± 8	26

Since the indentations were placed at regular intervals and the number of indentations was large, this value of 15 per cent represents an approximate value for the macro areas of a tooth that are harder or softer than the average values reported.

DISCUSSION

The results presented for the hardness of enamel sections (KHN average 343) are in general agreement with those of Caldwell, Muntz, Gilmore, and Pigman[10] on intact enamel surfaces (KHN average 380), which indicates that the inner enamel is of comparable hardness to surface enamel. The range of hardness values obtained in this research was 272 to 440 compared to 250 to 500 for the former investigation. It should be remembered that approximately 15 per cent of the measurements of this research were discarded for being outside the limits of experimental error or this range would be larger. The existence of these areas having large deviations in hardness from the average is substantiated by the work of Gustafson and Kling[8] on the hardness of enamel and its relation to histologic structure.

A number of workers[3, 6, 9, 10] have reported that the enamel outer surface is harder than the inner enamel and that the hardness decreases from the outer edge to the dentinoenamel junction. Others[8, 13] have found no difference in hardness from the dentinoenamel junction to the outer surface. In the present study, the distance from the first indentation to the enamel surface was approximately 0.05 mm. and, therefore, the first indentation does not actually represent the enamel surface. The data do show, however, that no significant differences in hardness existed from the dentinoenamel junction to the outer enamel edge. The most reasonable explanation for the various conclusions regarding the hardness trend in enamel has been proposed by Gustafson and Kling.[8] They have shown that different hardness trends could be observed by variation of the direction of a series of indentations in a single tooth section.

Hodge and McKay[6] reported that dentin near the root was softer than near the crown of a tooth (Bierbaum Nos. 130 and 140, respectively). This trend was not observed in this study but would be difficult to establish since the per cent of variation reported above is about 7.5 per cent, which is of the same order of magnitude as the standard deviation reported in this paper.

SUMMARY AND CONCLUSIONS

The Knoop hardness numbers were determined for human enamel and dentin on sections of mature, freshly extracted, noncarious teeth. The over-all average hardness value for enamel and dentin for all teeth was 343 ±23 and 68 ±3, respectively.

Approximately 15 per cent of the hardness values obtained on both enamel and dentin had such large deviations from the average that they must be considered to be outside the range of experimental error and represent areas having exceptionally high or low hardness.

No definite trend was detected in the hardness of enamel from the dentino-enamel junction to the outer surface or from the crown to the cervical margin. Likewise, no trend in hardness was observed in the dentin from one section of a tooth to another.

The greatest variation in the hardness in enamel was found just below the occlusal surface, although the average value for this type of section was not significantly different from the average values for other sections.

The assistance of C. R. Norris and G. G. Wepfer in determining the hardness values is gratefully acknowledged.

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THE SENSITIVITY OF HUMAN DENTIN

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A SENSATION of pain can be evoked when dentin is subjected to a variety of stimuli, but the peripheral mechanism of this sensitivity has not yet been elucidated. The many workers who examined this problem from a histologic standpoint were not in agreement in their conclusions. Bernick[1] has described nerve fibers in the predentin but not extending to the dentin. Cocker and Hatton[2] found fibers extending through the dentin halfway to the amelodentinal junction and lying in the ground substance rather than the tubules. Fernhead[3] produced convincing evidence for the presence of beaded fibrils in the dentinal tubules but even these have been traced only about halfway to the amelodentinal junction. It has thus been impossible as yet to provide a satisfactory histologic basis for the sensitivity of dentin, especially in the amelodentinal region which is said to be a very sensitive part of the tooth.

It was decided to attempt a functional approach to the problem of sensation in dentin by stimulating exposed dentin with various agents chosen on the basis of clinical experience and previous physiologic experiments on pain elsewhere in the body. Sweet substances can cause dental pain and for this reason sucrose was chosen as a test solution. Two concentrations, 1 M and 2 M were taken. The experiments of Armstrong, Dry, Keele, and Markham,[4] demonstrated that a variety of substances caused pain when applied to the base of a blister where nerve endings were thought to lie. If such substances also caused pain when applied to exposed dentin this would be strong evidence in favor of the view that pain was due to the stimulation of nerve endings at or close to the exposed surface of the dentin. On the basis of the above experiments,[4] acetylcholine and potassium chloride were chosen, but in concentrations of 10^{-2} Gm. per milliliter and 1 M, respectively, which concentrations were considerably higher than those used by Armstrong, Dry, Keele, and Markham.

METHOD

The first experiment of this investigation was planned for 24 subjects. Thirty-one individuals submitted to a preliminary test; 29 were males and 2 were females and their ages were between 18 and 31 years. In all the experiments, dentin for testing was deliberately exposed by cutting from the occlusal surface of a tooth. Although dentin exposed at the neck of a tooth

Received for publication Dec. 12, 1957.
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by wear or with age changes is known to be sensitive, it was considered that the use of this would introduce unnecessary variables. The cavities were cut in molar or premolar teeth which on superficial examination appeared to be only slightly affected by dental caries. Sometimes extensive caries was found after cavity preparation had commenced, but it was decided to use all the teeth offered. No anesthetic was used and the cavities were cut with steel burs run at a slow speed with no attempt to exclude saliva. After preparation to a depth required by the extent of caries, each cavity was rinsed with warm water and dried with cotton wool. The test solution was inserted into the cavity on cotton wool and the subject was told to start a clock, which he stopped as soon as he felt anything or after 30 seconds if he felt nothing. After rinsing and drying, the next solution was applied. All the solutions were kept in a water bath at 37° C. A preliminary test of reactivity was made by the application to the dentin of cotton wool soaked in ice-cold water, and this preceded all the other solutions. No indication was given to the subject that there was anything unusual about this solution, but if he failed to respond to it, the experiment was terminated, and he was excluded from the group. Using the ice-cold water test to indicate reactivity, 6 males and 1 female were excluded, leaving 24 subjects for testing.

Each subject submitted to tests with 2 arrangements of the series of 4 solutions twice in succession. For the purpose of arranging the order of application, the solutions were designated by the letters A, B, C and D, and the 24 possible permutations of these letters were set out. A randon allocation of 2 of these permutations was made to each subject. Thus the testing order for the first subject was DCAB BCAD, signifying 2 M sucrose, 1 M KCl, 1 M sucrose, acetylcholine, acetylcholine, 1 M KCl, 1 M sucrose, 2 M sucrose. The random allocation of the permutations of testing order ensured that every solution was administered twice to every subject (once in each successive set of 4 applications) and, for the experiment as a whole, the order of administration could have no effect on the results with different solutions. The operator had no knowledge of the order in which the solutions were applied, since they were dispensed by an assistant, and the subjects did not know how many different solutions were to be used, nor how frequently they were to be applied.

RESULTS

Experiment 1.—

Table I shows the number of responses given by the subjects to the 4 solutions and it is clear that 2 M sucrose scored the highest number of responses, with 25 responses, compared with 10, 6, and 3 for 1 M sucrose, acetylcholine, and 1 M KCl, respectively. These differences can be shown statistically to be highly significant ($\chi^2 = 26.0$, n = 3, $< P$ 0.0001). The high score of 2 M sucrose contributed the main share of variation, and there is not enough evidence to show that there was a real difference between the other 3 solutions. The design of the experiment ensured that this result could not have been accounted for by 2 M sucrose having been administered in any special position relative to

TABLE I. THE NUMBER OF RESPONSES TO 2 TESTS WITH A SERIES OF 4 SOLUTIONS APPLIED TO THE DENTIN OF 24 SUBJECTS

SUBJECT	1 M SUCROSE	2 M SUCROSE	ACETYLCHOLINE 10^{-2} GM./ML.	1 M KCl
1	0	2	0	0
2	0	0	0	1
3	1	1	0	0
4	0	1	0	1
5	1	1	0	0
6	0	0	1	0
7	0	2	0	0
8	0	0	0	0
9	0	1	0	0
10	0	0	0	0
11	0	2	0	0
12	0	0	0	0
13	1	1	1	1
14	1	1	0	1
15	2	1	0	0
16	1	2	0	0
17	0	2	0	1
18	1	2	0	0
19	0	1	0	0
20	0	1	0	0
21	1	0	0	0
22	0	1	1	0
23	1	1	0	0
24	0	2	0	1
Total	10	25	3	6

the other solutions. Analysis of the results according to the order of administration failed to demonstrate any appreciable trend in the sensitivity as the experiment proceeded. In fact there were 21 responses in the first 4 tests (adding up the results from each subject) and 23 among the second 4, a finding which could easily have arisen by chance.

After every response the subject was asked to dictate a description of the sensation he had experienced into a tape recorder. As might be expected, the descriptions showed considerable variety. For example, there were descriptions such as "a faint tingling," "a growing pain," "a sharp, shooting pain," "a dull ache," "a sudden feeling of heat," "an electric shock," etc.

The results are in agreement with the common experience that sugar can cause pain in teeth, but as yet it cannot be said whether this is due to the sweetness or some other characteristic of the substance. It seemed likely that osmotic activity might be a factor in causing pain and this was one reason for choosing 1 M KCl and 2 M sucrose, both having approximately the same depression of freezing point. However, 1 M KCl produced far fewer responses than 2 M sucrose (see Table I).

With such variables as the extent of the dental caries, secondary dentin, translucent zone dentin, and dead tract dentin, the difference between individuals is not surprising. There is no obvious explanation for the fact that although 18 subjects responded to 2 M sucrose, only 7 responded to both applications of this solution. The pairs of results with each solution can be classified

as "Agree Positive," "Agree Negative," and "Disagree." From Table I the results are summarized in this classification as follows:

	1 M Sucrose	*2 M Sucrose*	*Acetylcholine*	*1 M KCl*
Agree Positive	1	7	0	0
Agree Negative	15	6	21	18
Disagree	8	11	3	6

The large number of disagreements shows that our experiment does not demonstrate that there is any common characteristic among subjects or cavities which might influence the result. This is less easily seen with the solutions with a low response rate, than with the 2 M sucrose for which there were 11 disagreements and 13 agreements. More rigid control of variables in future experiments might make it possible to demonstrate uniformity. In addition, it seems that no subjects can be picked out with a high or low threshold to the 4 solutions in general; the total number of "positives" scored by each subject is as would be expected if the 44 "positives" were distributed at random among the 24 subjects.

If pain from dentin exposed in cavity preparation or involved in dental caries depended on the direct stimulation of nerve elements in the dentin, many more responses than were recorded with KCl and actylcholine would have been expected. In addition to factors such as the extent of caries, secondary dentin, etc., which might prevent a response, it is possible that damage caused in cavity preparation, or the accumulation of debris at the cut ends of the dentinal tubules prevented contact between the stimulating solution and the receptor mechanism. It is hard to understand, however, why such factors should operate less in the case of the sugar solution than in the case of the KCl and acetylcholine. There seems to be no relationship between sensitivity to these chemical stimuli and sensitivity to ice-cold water and instrumentation, since all the subjects responded to cold and most of them felt pain during cavity preparation and scratching the dentin with a probe.

Experiment 2.—

Since 2 M sucrose gave more responses than 1 M sucrose, it seemed likely that a further increase in the concentration would result in more responses and this was supported by results of a comparative test on 5 subjects with cavities prepared in the manner already described. Sucrose, 2 M, and sugar syrup (sucrose + invert sugar, 80.25 per cent) were applied twice in each subject. Out of 10 applications there were 8 responses to the syrup but only 1 response to the 2 M sucrose.

Experiment 3.—

In order to reduce the number of variables associated with a random sample of teeth with carious and noncarious dentin, another series of 11 teeth was taken in which it was only just possible to make a probe stick by applying it with very firm pressure to the fissure. In none of the 11 cavities of this series was there any evidence either from the hardness of the dentin or its color to suggest that it was carious. To meet the criticism that the damage caused in

cavity preparation might interfere with the function of a receptor mechanism for pain in the dentin, the cavities were prepared under a cold-water jet using a tungsten carbide bur revolving slowly. According to Marsland and Shovelton,[5] this technic results in no obvious histologic damage to the pulp and although there is no evidence concerning the state of the contents of the dentinal tubules it is unlikely that dentin can be exposed with less damage than by this method. In this series, the ice-cold water test was omitted because all the subjects felt pain during cavity preparation.

The procedure in this group of 11 subjects differed from the first experiment in some respects. To diminish the risk of missing a delayed response, 1 minute instead of the previous 30 seconds was allowed for stimulation if no response occurred earlier. Syrup only was used for testing and to make sure of removing the test solution the cavity was washed with water for 2 minutes before the next application. To test the reproducibility of the response, 6 tests were made in succession and the latent period between stimulation and response was recorded, but if a subject failed to respond to 2 consecutive stimulations, the experiment was discontinued. As before, the subjects did not know how often the solutions were to be applied nor whether they were all the same or not. The results are shown in Table II.

TABLE II
THE LATENT PERIOD BETWEEN STIMULATION AND RESPONSE IN DENTIN STIMULATED WITH SYRUP TO A MAXIMUM OF 6 TIMES IN SUCCESSION*

SUBJECT†	STIMULUS					
	1	2	3	4	5	6
2	5.0	2.0	3.0	3.0	4.0	2.5
3	6.0	5.0	6.5	3.5	6.0	8.0
4	2.5	2.5	9.5	8.0	25.0	34.0
8	5.0	18.0	--	--		
9	17.5	--	--			
11	13.0	1.5	--	--		

*No response after 1 min. is shown by dash. Two consecutive failures terminated the test.

†Subjects 1, 5, 6, 7, and 10 had no response after 1 minute the first 2 times tested and no further tests were made. Latent period in seconds.

The experience of 1 subject in the group of 24 in Experiment 1 suggested a further approach. This subject failed to respond to any of the solutions applied to a small cavity in a tooth only slightly affected by caries of the enamel. The cavity was filled with amalgam without lining and during the following 3 weeks the subject complained of pain associated with temperature change and also noticed that sweet foods caused pain in that area of the mouth. Inspection showed no obvious leakage round the margins of the amalgam, but there were other pits in the enamel which might have provided diffusion pathways to the dentin rendered hypersensitive by the treatment to which it had been subjected. There was no evidence of sensitivity at the cervical margin of this tooth. The amalgam was removed and the dentin was extremely sensitive to instrumentation. In addition to the 4 solutions used in the first experiment, syrup was applied. Both applications of syrup caused an immediate painful response which was of considerable severity and lasted after the removal of the cotton

wool until the cavity was rinsed. The response to 2 M sucrose was less vigorous and the pain had begun to die away before washing commenced. The first application of 1 M sucrose, acetylcholine, and KCl caused pain almost immediately but it died away before washing began. There was no response to the second application of these 3 solutions.

TABLE III
THE EFFECT OF LEAVING CAVITIES FILLED WITH GUTTA PERCHA FOR 1 WEEK ON THE SENSITIVITY TO SYRUP*

SUBJECT†	STIMULUS					
	1	2	3	4	5	6
1 (a)	--	--				
(b)	3.0	4.0	4.5	5.0	12.5	23.5
3 (a)	5.0	18.0	--	--		
(b)	2.0	4.0	6.5	5.0	3.5	6.5
4 (a)	17.5	--	--			
(b)	5.0	1.0	1.5	5.0	1.0	5.0
5 (a)	13.0	1.5	--	--		
(b)	3.5	0.5	Immediate response	Immediate response	2.0	Immediate response

*(a) The latent periods on testing immediately after cavity preparation.
(b) Values obtained after gutta percha filling for 1 week.
A maximum of 6 stimulations was made. No response after 1 min. is shown by a dash. Two consecutive failures terminated the test. Latent period in seconds.
†Subject 2 gave no response after 1 minute in tests a and b.

TABLE IV
THE EFFECT OF LEAVING CAVITIES FILLED WITH GUTTA PERCHA FOR 1 WEEK ON THE SENSITIVITY TO SYRUP, ACETYLCHOLINE, AND KCl
(TWO STIMULATIONS WERE MADE WITH EACH SOLUTION)

		RESPONSES TO TWO STIMULATIONS WITH		
		SYRUP	ACETYLCHOLINE 10^{-2} GM./ML.	1 M KCl
Subject 1	After cavity preparation	0	0	0
	1 week later	2	0	1
Subject 2	After cavity preparation	1	0	0
	1 week later	2	0	0
Subject 3	After cavity preparation	1	0	0
	1 week later	2	1	1
Subject 4	After cavity preparation	1	0	0
	1 week later	2	0	1
Subject 5	After cavity preparation	0	0	1
	1 week later	2	0	0

Experiment 4.—

In order to obtain further data of the type just described, 5 cavities in subjects of Experiment 3, which either completely failed to respond or responded to less than half the applications of syrup immediately after cavity preparation, were filled with gutta percha and left for 1 week before retesting. Table III shows the latent periods between stimulation and response (a) immediately after cavity preparation, and (b) after leaving the cavity filled with gutta percha for 1 week. In addition, 5 cavities in an additional group of subjects were tested immediately after preparation, with 2 applications of syrup, acetylcholine, and KCl. The tests were repeated 1 week later, the cavities having been filled with gutta percha for that period. Following this treatment

the dentin in both of these groups was very sensitive to cold and to scratching with a probe. In addition, as is shown in Tables III and IV, there was an obvious increase in sensitivity to syrup in 9 out of 10 subjects, whereas the results with acetylcholine and KCl are far less striking. One of 5 subjects in Table IV responded to 1 out of 2 applications of acetylcholine to a cavity previously filled with gutta percha; all 5 subjects failed to respond to this solution immediately after cavity preparation. There were 3 responses to 10 applications of 1 M KCl after gutta percha treatment compared with 1 immediately after cavity preparation.

Experiment 5.—

The last group of tests concerns the effect of a local anesthetic solution applied to the dentin. Mongar[5] found that the application of 2 per cent Lignocaine to the blister-base preparation for 30 seconds produced almost complete anesthesia. It was decided to apply 2 per cent Lignocaine for 90 seconds, dry the cavity, and to test with syrup immediately without previous washing. The difficulty in such experiments was to be certain that an apparently successful application of anesthetic was not merely a decrease in sensitivity of the dentin. The lengthening of the latent period of Subject 4 in Table II and the failure to elicit more than 2 responses in Subjects 8, 9, and 11 in the same group suggest a progressive decline in sensitivity. Preliminary tests with local anesthetic were inconclusive for this reason, and an attempt was made to get over this difficulty by using the same tooth for 2 separate tests. Two cavities were cut in an occlusal fissure which would normally have been opened as a single cavity. In the first cavity a series of 6 tests was made with syrup according to the routine already described, and the latent periods were recorded.

TABLE V
THE LATENT PERIODS BETWEEN STIMULATION WITH SYRUP AND RESPONSE IN 2 CAVITIES IN THE SAME TOOTH*

		STIMULUS					
		1	2	3	4	5	6
Subject 1	Cavity 1	5.0	2.0	3.0	3.0	4.0	2.5
	Cavity 2	15.0	17.0	13.0	6.5	26.0	8.0
Subject 2	Cavity 1	6.0	5.0	6.5	3.5	6.0	8.0
	Cavity 2	6.0	3.0	5.0	8.0	10.0	18.5

*Six successive stimulations were made and Lignocaine was applied for 90 sec. to cavity 2 between the third and fourth application of syrup. Latent period in seconds.

This provided a control and when it was completed the cavity was filled. The second half of the fissure was then cut, leaving a bridge of enamel and dentin separating the 2 halves. The series of 6 tests with syrup was repeated on this cavity but with the introduction of a pledget of cotton soaked in 2 per cent Lignocaine for 90 seconds between the third and fourth application of syrup. Unfortunately, owing to the small number of successful stimulations with syrup on dentin not exposed previously to gutta percha, only 2 subjects were suitable for this test. The results shown in Table V indicate that 2 per cent Lignocaine was unsuccessful. Lignocaine was also tested on the cavities previously exposed to gutta percha for 1 week. In the group of 5 subjects dealt

with in this way, 2 per cent Lignocaine was applied for 90 seconds after testing with syrup. The dentin was then retested with syrup and in all 5 subjects the response was undiminished in vigor.

DISCUSSION

The simplest hypothesis to account for the peripheral mechanism of the sensation of pain from dentin is that it depends upon the direct stimulation of nerve elements (endings or fibers) in the tubules. However, Fernhead[3] found that nerve fibers could be traced only part of the way through the thickness of the dentin. An alternative hypothesis which fits more closely with the histologic evidence is that some or all stimuli act by means of a disturbance transmitted through the dentinal tubules either to nerve elements which terminate at some point in the tubule well short of the amelodentinal junction, or to the pulpal surface which is profusely innervated.

When a probe or excavator is drawn firmly across the surface of the dentin this is usually painful. If this sensation is due to direct stimulation by touching or cutting nerve elements, then substances known to evoke pain elsewhere in the body should be capable of causing pain when applied to exposed dentin. The failure of 2 of such agents chosen for this investigation to excite pain in dentin is evidence against the existence of nerve elements close to the ends of the exposed dentinal tubules. The alternative hypothesis stated above places the nerve elements at some level short of the amelodentinal junction or in the pulp, and it is possible that stimulating solutions applied to the dentin in shallow cavities would be unable to reach these deeply placed structures. If this were so, shallow cavities would generally be less sensitive than deep cavities, but no evidence for this has been found in this investigation. The cavities in the first series of 24 subjects were of varied depth and some of them must have extended far enough to involve any nerve elements which might be present deep in the dentinal tubules, but the responses recorded with acetylcholine and 1 M KCl were not confined to the deepest cavities. It might be that the carious process and reactions to it inactivated these structures in some way making them unresponsive to acetylcholine and 1 M KCl, but if this were so then there must be an alternative mechanism for the pain sensation, since all the teeth of this group responded to cold, many were painful on instrumentation, and some responded to the sugar stimulus. Furthermore, in every subject in the first series, the cavity was extended to involve what appeared to be sound dentin and it seems unlikely that nerve elements were exposed as seldom as the 3 responses to acetylcholine and 6 responses to 1 M KCl suggest. Only 1 subject responded to both acetylcholine and 1 M KCl, but even this was to 1 of the 2 applications of each solution.

If the sensation of pain from dentin requires the direct stimulation of nerve elements at the exposed surface, the application of a local anesthetic would be expected to abolish pain whatever the stimulus. The failure to achieve anesthesia with local anesthetic suggests that nerve elements are not easily accessible to solutions applied to the dentin.

If caries and damage during cavity preparation were responsible for the failure of acetylcholine and 1 M KCl to cause pain, they did not prevent a response to other stimuli. Caries and damage cannot be held responsible either for the almost complete failure of these solutions or for the small number of responses to syrup in the series of experiments summarized in Tables II and IV. Since the cavities in this group were in teeth hardly affected by caries and were therefore very shallow, it is possible that the stimulating solutions failed to reach deeply placed nerve fibers or endings either in the tubules or at the pulpal surface. However, when these cavities were filled with gutta percha for 1 week, the sensitivity to syrup was greatly enhanced while leaving the sensitivity to acetylcholine and 1 M KCl almost unaltered. It is unlikely that this procedure brought nerve elements closer to the surface and it seems possible that the pulpal hyperemia known to be associated with gutta percha fillings[7] is a contributory factor to dentin sensitivity in these experiments.

CONCLUSIONS

These experiments indicate that the sensation of pain from dentin does not depend on direct stimulation of nerve elements at the cut ends of the dentinal tubules. Alternatively, the stimuli may act by means of a disturbance transmitted through the dentinal tubules. The evidence leans in favor of the view that the receptor mechanism is in the pulp rather than in the dentin.

Our thanks are due to the subjects for their cooperation and to the Nuffield Foundation for a grant to one of us (D. J. A.) which has supported this work.

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SOME INFLUENCES AFFECTING REPAIR OF THE EXPOSED PULPS OF DOGS' TEETH

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NUMEROUS investigators have demonstrated that the pulp of human and animal teeth has the ability to repair itself following exposure. Abundant histologic evidence of the stimulatory influence of calcium hydroxide on the formation of secondary dentin is available. The literature on this subject has been extensively reviewed by Euler,[4] Nyborg,[11] and many others.

Other factors and materials influencing the repair of exposed dental pulps have also been investigated. These influences include numerous drugs whose purposes were to stimulate or sterilize or both, blood clotting, removal of greater or lesser amounts of pulp tissue, cautery, and cajolery. Quigley[14] has comprehensively reviewed the investigations in these areas. Most investigators have assumed that if a bridge of secondary dentin was produced, the pulp was completely repaired and no subsequent degeneration could or would occur.

The purposes of our experiments on the exposed pulps of dogs' teeth were to study the influences of drugs, alkalinity, time, and blood clot formation on pulp repair. Besides studying pulp reactions subjacent to the region of exposure and drug placement, observations were also made in the radicular and periapical regions.

PROCEDURES

Three young, healthy dogs were operated upon under general anesthesia. The exact ages of the dogs were unknown, but each was believed to be between 1 and 2 years old. Cavities were prepared, under aseptic conditions, on the buccal surfaces of 18 teeth of each of 2 dogs. On a third dog, similar cavities were prepared on 16 teeth. Thus, a total of 52 teeth were operated upon. After suitable retention form had been completed for each cavity, the pulp was exposed near the gingival floor with a sterile No. 4 round bur. The head of the bur was permitted to plunge into the pulp. As soon as the pulp had been entered, the bur was withdrawn. This was usually followed by hemorrhage. However, occasionally hemorrhage did not take place until the exposure site was touched by an exploring tine. In half of the number of experimental teeth of each animal, the blood was wiped out of the cavity with 1 or more sterile cotton pellets. A medicament, which was dissolved or suspended in water or blood, or sterile saline solution, was then applied to the exposed pulp. After 1 minute the exposures were covered with sterile asbestos fibers, the dentin was wiped dry with sterile cotton pellets and the cavities were filled with amalgam.

Received for publication Dec. 12, 1957.

In the other half of the number of teeth involved in the experiment, the same procedures were employed to expose the pulps. A blood clot was then allowed to form before any medicaments were placed over the exposure site. In one tooth of each dog, no medication was applied after the blood had clotted. In 2 teeth, a medicament in dry amorphous form was applied. All the other exposures were suspended or dissolved in sterile distilled water. All exposures were then covered with sterile asbestos fibers and the cavities were filled with amalgam.

Drugs Tested.—The following medicaments were applied to the pulp exposures: (1) Various calcium salts, dissolved or suspended in sterile, distilled water. These salts were calcium chloride (397.0 mg. %) pH 6.9, calcium carbonate (270.0 mg. %) pH 9.4, tricalcium phosphate (279.0 mg. %) pH 6.4, and calcium hydroxide (200.0 mg. %) pH 12.0; (2) an aqueous solution of ammonium hydroxide, buffered to pH 12.0; (3) normal saline solution, pH 4.5; (4) an aqueous solution of 250,000.0 units per milliliter of potassium penicillin G, pH 6.8; (5) an aqueous suspension of alkaline phosphatase in concentration of 100.0 units per milliliter, pH 8.2; and (6) dry amorphous alkaline phosphatase, 100 units per milligram.

One drop of solution (approximately 0.05 ml.) was applied to each cavity. However, the amount of solution available to the exposed pulp varied with the volume of the cavity. In small cavities the volume of one drop of solution was larger than the volume of the cavity; consequently, some of the solution overflowed the cavity walls. Where dry alkaline phosphatase was used, the exact amount was not measured. The amount of powder placed over the exposure was that amount necessary to dissolve in the blood or serum present over the exposure site.

One animal was sacrificed within 7 days. The other 2 were sacrificed at intervals of 30 and 90 days following the aforementioned operative procedures. The animal was sacrificed by perfusion of formalin through cannulae placed bilaterally in the common carotid arteries. Following death, the animals were decapitated and each tooth was separated from the jawbone with a band saw. The teeth were then each placed in individual bottles of 10 per cent formalin. Following decalcification, frozen sections were prepared. These were stained with hematoxylin and eosin for microscopic study.

FINDINGS

Seven Days.—Although there were some variations in intensity, reactions of the pulp 7 days after exposure were similar regardless of whether or not drugs were placed over the exposed pulps, what kind of drugs were employed, or whether or not a blood clot was allowed to form before covering the exposure. The changes to be described were typical of those seen in most of the teeth. However, every tooth did not necessarily present all of the reported findings. The plane of sectioning had an important influence over the kind of reactions which were discernible in each slide. It was often found that sections in one plane showed mild pulp reaction, whereas alterations in the plane of sectioning of the same tooth produced findings which were significantly more severe.

In most teeth, dentin chips or fragments had been pushed into the pulp as a result of the operative procedure. In some instances, especially following the use of alkaline phosphatase, calcium carbonate, calcium phosphate, and calcium hydroxide, these chips were welded together with pink-staining dentinoid material. This was particularly striking with the use of alkaline phosphatase (Fig. 1). A similar reaction was also found in those pulps where no drugs were used, but to a lesser degree. Histiocytes were present in abundance around the dentin fragments. Other cells oriented along the dentin chips had the appearance of fibroblasts. Especially where alkaline phosphatase had been used, cells had become oriented in a palisade arrangement along the eosinophilic dentinoid material giving the appearance of new odontoblasts (Fig. 2). These odontoblast-like cells were not, however, arranged uniformly all around the dentinoid material in a number of instances. Many regions of dentin were not lined with cells at all.

In the region of exposure, the odontoblasts had been destroyed. The odontoblasts, incisal or occlusal to the exposure site, were disturbed and had lost their orientation. This was especially noticeable on the dentinal wall opposite that of the exposure side. Here the odontoblastic layer was compressed and the nuclei in many instances had undergone karyorrhexis. Toward the radicular portion of the pulp, the odontoblastic layer was compressed and shrunken. This alteration was present for some distance before the odontoblasts assumed a normal morphologic appearance. In many instances the odontoblasts had been replaced by fibrous connective tissue. Polymorphonuclear leukocytes were often found lying against the predentin in those regions where the odontoblasts had degenerated.

The pulp was destroyed in the region of the exposure. Hemorrhage had taken place into the body of the pulp and scattered erythrocytes were found in some instances for quite a distance into the radicular portion of the pulp. Surrounding the exposure site was a dense infiltration of polymorphonuclear leukocytes, indicative of a large coronal abscess (Fig. 3). Although the remainder of the pulp retained its architecture, it was infiltrated for a considerable distance with neutrophils, lymphocytes, and large macrophages. The infiltration was noticeably heavier in those teeth in which the pulp had been treated with calcium chloride and ammonium hydroxide. The blood vessels in the remaining pulp tissue were dilated and hyperemic in all instances.

Thirty Days.—The effect of the exposure was apparently reflected in the dentin by a disturbance in calcification at the time of exposure. This was evidenced by a deeply basophilic line in the dentin opposite the injured odontoblasts (Fig. 4). The pulps in most instances were either necrotic or converted to granulation tissue (Table I).

The pulps were necrotic following the formation of a blood clot and the placement of the following solutions of chemicals over the exposures for 1 minute: alkaline phosphatase, ammonium hydroxide, and calcium chloride. Granulomas were present in the apical tissues of these teeth. The pulps also became

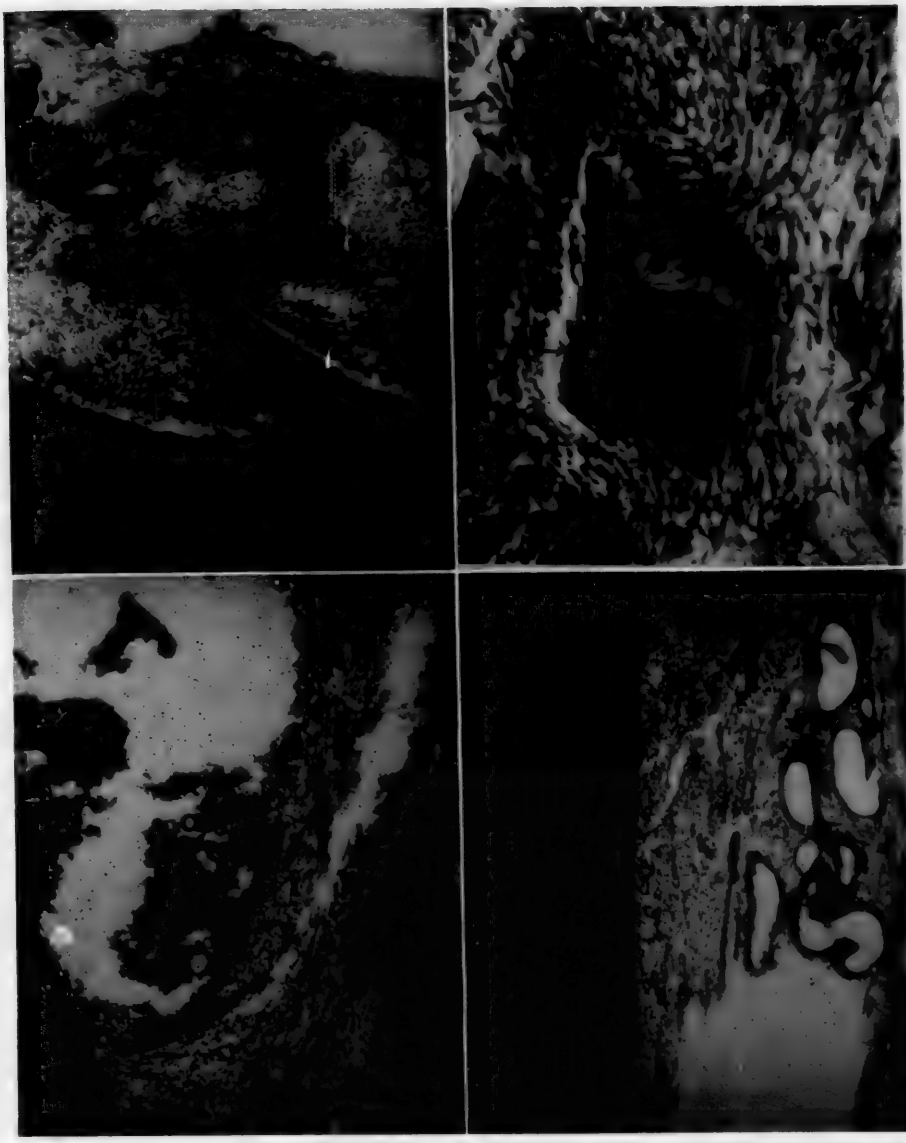

Fig. 1.—Buccolingual section of lower right carnassial, 7 days after operation. Pulp exposure was treated with dry alkaline phosphatase after blood had clotted. Dentin chips (*c*) pushed into pulp (*p*) as result of operative procedure, welded together with dentinoid material. (Orig. mag. ×83.)

Fig. 2.—Labiolingual section of lower left canine, 7 days after operation. Pulp exposure was treated with aqueous suspension of alkaline phosphatase without waiting for a blood clot to form. Apparently new odontoblasts (*o*) oriented around dentin fragment (*c*) found lying in the pulp (*p*). (Orig. mag. ×270.)

Fig. 3.—Labiolingual section of upper left third incisor, 7 days following operation. Pulp exposure was treated with aqueous solution of calcium chloride without waiting for a blood clot to form. Dense infiltration of inflammatory cells, indicative of coronal abscess (*a*). Hemorrhage in body of pulp (*h*). Remainder of pulp infiltrated with inflammatory cells (*i*). (Orig. mag. ×270.)

Fig. 4.—Labiolingual section of upper right canine, 28 days following pulp exposure. Treatment was with aqueous suspension of calcium hydroxide without waiting for blood clot to form. Calcio-traumatic band (*b*) evident in dentin (*d*) indicating disturbance of calcification. (Orig. mag. ×83.)

TABLE I
PULP AND APICAL TISSUE REACTIONS 30 AND 90 DAYS FOLLOWING TREATMENT OF EXPOSED PULPS

TREATMENT	NECROTIC PULP		NECROTIC PULP AND APICAL GRANULOMA		INTACT RADICULAR PULP TISSUE WITH INFLAMMATORY CELL INFILTRATION		CALCIFIC DEPOSITS OVER PULP EXPOSURE; REMAINING PULP TISSUE NORMAL	
	30 DAYS	90 DAYS	30 DAYS	90 DAYS	30 DAYS	90 DAYS	30 DAYS	90 DAYS
Without Clot								
Normal saline	x							x
Amorphous alkaline phosphatase*		x		x				
Aqueous penicillin		x		x	x			
Alkaline phosphatase suspension					x	x		
NH_4OH	x	x		x				
$Ca_3(PO_4)_2$	x	x						
$CaCl_2 \cdot 2H_2O$					x	x		
$CaCO_3$		x			x			
$Ca(OH)_2$							x	x
With Clot								
No medication		x		x	x			
Dry alkaline phosphatase*		x		x				
Aqueous penicillin		x		x	x			
Alkaline phosphatase suspension	x		x			x		
NH_4OH	x	x	x	x				
$Ca_3(PO_4)_2$		x		x	x			
$CaCl_2 \cdot 2H_2O$	x	x	x	x				
$CaCO_3$		x		x	x			
$Ca(OH)_2$†						x		

*Not applied in 30-day specimen.
†30-day specimen was discarded because tooth was fractured in situ.

necrotic following treatment with the following chemical solutions or suspensions for 1 minute without waiting for a blood clot to form after pulp exposure: normal saline, calcium phosphate, and ammonium hydroxide.

In those instances where a blood clot was permitted to form and drugs were then placed on the pulp wound, the radicular pulp tissue was found to be intact following the placement of potassium penicillin, ammonium hydroxide, calcium phosphate, and calcium carbonate solutions or suspensions. Intact radicular pulp tissue was also found in the tooth which was not medicated. Where the drugs were placed without waiting for the blood clot to form, intact radicular pulp tissue was found after the use of penicillin, calcium chloride, calcium carbonate, and alkaline phosphatase. In all instances where intact radicular tissue was found, however, this tissue was infiltrated in varying degrees with erythrocytes, polymorphonuclear leukocytes, lymphocytes, plasma cells, and macrophages. Blood vessels in the intact pulp were uniformly hyperemic and congested.

In the pulp of the tooth treated with calcium hydroxide without waiting for a blood clot to form, a large amount of secondary dentin had been deposited around dentin fragments. The remaining pulp tissue was relatively normal.

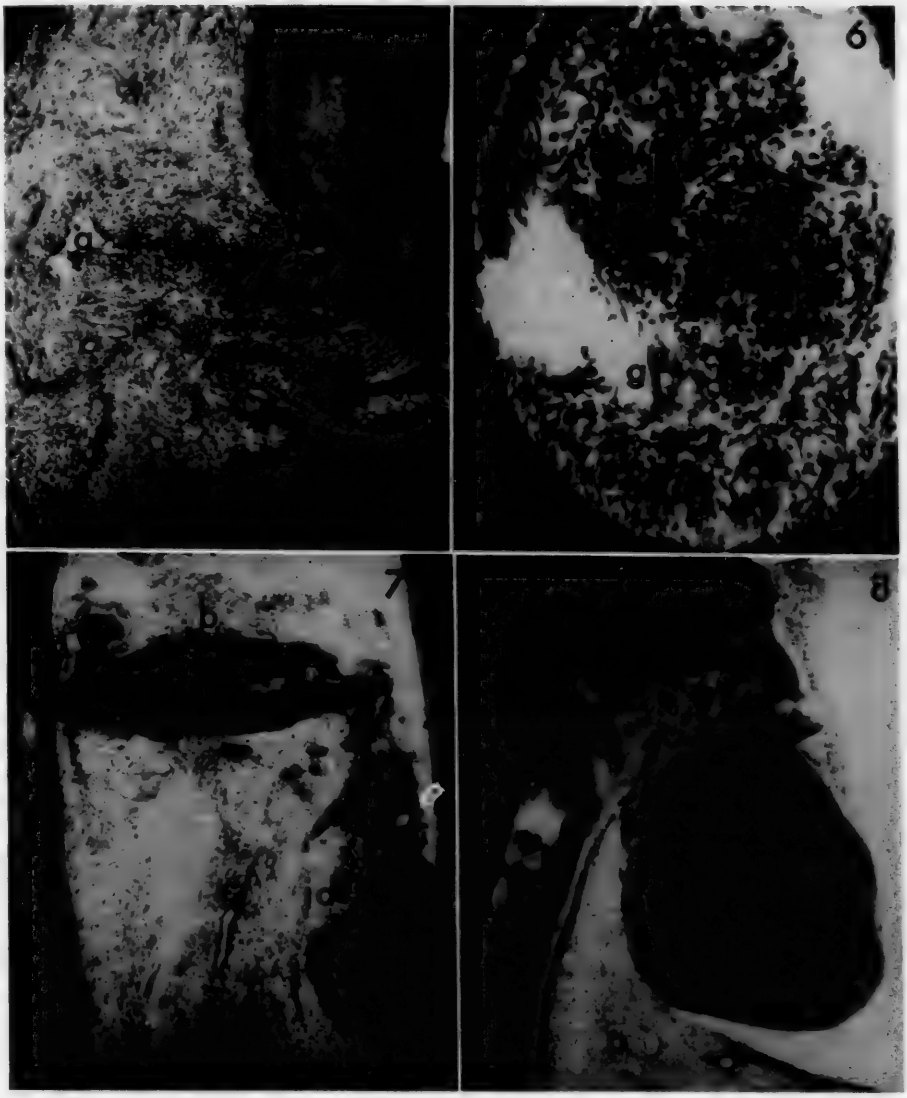

Fig. 5.—Buccolingual section of lower right third molar. Ninety days have elapsed since operation. The exposed pulp had been treated with an aqueous solution of potassium penicillin without waiting for a blood clot to form. Necrotic pulp at apical foramen (*f*). Large apical granuloma (*g*) surrounds the root apex. (Orig. mag. ×83.)

Fig. 6.—Buccolingual section of lower right second molar, 90 days after operation. Pulp exposure was treated with aqueous suspension of calcium hydroxide after a blood clot had formed. Pulp has been converted into granulation tissue (*g*) which is heavily infiltrated with inflammatory cells (*i*). (Orig. mag. ×270.)

Fig. 7.—Labiolingual section of lower right cuspid, 90 days following operation. Pulp exposure was treated with physiologic saline solution without waiting for a blood clot to form. "Bridge" of irregular dentin is present (*b*), sealing off exposure site. Irregular dentin also present on axial wall of radicular pulp (*id*). (Orig. mag. ×83.)

Fig. 8.—Labiolingual section of upper right cuspid, 90 days after operation. The exposed pulp was treated with an aqueous suspension of calcium hydroxide without waiting for a blood clot to form. Tremendous amount of irregular dentin (*id*) surrounding dentin chips. Remainder of pulp (*p*) relatively normal. (Orig. mag. ×83.)

The tooth which was treated with calcium hydroxide after a blood clot had formed was fractured in situ sometime during the experiment. It was subsequently discarded.

Ninety Days.—Where a clot had been permitted to form before drugs were placed on the exposed pulp, a necrotic pulp was found after treatment with potassium penicillin, ammonium hydroxide, calcium phosphate, calcium chloride, calcium carbonate, and dry alkaline phosphatase. A necrotic pulp was also discovered in the tooth in which the pulp had been exposed but not treated with drugs. Periapical granulomas were also discovered in the sections of each of these teeth (Fig. 5). The remaining pulp tissue was found to be intact but infiltrated with various inflammatory cells or converted into granulation tissue following the application of alkaline phosphatase suspensions and also after the use of calcium hydroxide (Fig. 6). In no instance was normal pulp tissue discovered nor was there any evidence of the formation of a dentin bridge.

When drugs were placed for 1 minute on the exposed pulp without waiting for an intervening blood clot to form, necrotic pulps resulted in the teeth treated with potassium penicillin, calcium phosphate, ammonium hydroxide, amorphous alkaline phosphatase, and calcium carbonate. Apical granulomas were detected in the sections of the teeth treated with potassium penicillin, amorphous alkaline phosphatase, and ammonium hydroxide. Intact radicular pulp tissue, which was nonetheless infiltrated with inflammatory cells or converted into granulation tissue, was evident following the application of alkaline phosphatase and calcium chloride solutions. In 2 instances, normal pulp tissue was discovered under the exposure site. In both of these pulps there was a prolific deposition of secondary dentin and an atempt at formation of a dentin bridge. The medicaments which had been applied to these teeth were normal saline solution (Fig. 7) and calcium hydroxide (Fig. 8).

DISCUSSION

Within 7 days, the effects of the drugs themselves were secondary in importance to the traumatic effects of the pulp exposure. The pulps were exposed with a No. 4 round bur which, in the smaller teeth, caused the loss of most of the coronal portion of the pulp. The operation was thus more analogous to a pulpotomy than a pulp exposure in those teeth. This trauma of exposure was sufficient to cause degeneration of odontoblasts, disturb calcification of the dentin, and produce an acute abscess within the pulp around the exposure site. Reactions may have been aggravated by the heat produced during the cavity preparations.

The odontoblasts are exceedingly sensitive to irritation. They reacted to the trauma of pulp exposure by characteristic degenerative changes, such as loss of palisading, reduction in size, and pyknosis of nuclei, even at distances far removed from the original injury.

The dentin formed at the time of pulp exposure was also affected by the trauma. Calcification was disturbed, as evidenced by a deep bluish zone adjacent to the injured odontoblasts. This is probably the same type of reaction

as was noted by Silberkweit, Massler, Schour, and Weinmann,[21] in the dentin of rat incisors following operative procedures. They referred to this as a "calciotraumatic reaction."

The remaining pulp tissue was invariably heavily infiltrated with inflammatory cells and erythrocytes, regardless of whether or not a blood clot was allowed to form before the medicaments were placed over the exposure. Even though Englander, Massler, and Carter[3] implied important therapeutic significance to the formation of a blood clot prior to the application of pulp capping materials in human beings, there appeared to be no significant differences in dogs at the end of 7, 30, or 90 days between those pulps which were capped after a blood clot had formed and those upon which the medicaments were placed without waiting for a blood clot to form. A similar observation, following pulp exposures on hamsters' teeth, has been reported by Quigley.[15]

These experiments emphasize the futility of drug therapy for uninfected exposed pulps. Antibiotics are not immune from this indictment. Recovery or ultimate necrosis depends on the extent of cell destruction which has been caused by the pulp exposure. Extensive cell destruction, with resultant liberation of autolytic products, produced pulp necrosis and the formation of apical granulomas regardless of whether or not medication was applied to the pulp, what kind of medication (with the exception of calcium hydroxide), or whether or not a blood clot formed first. The additional toxic effect of the chemicals applied to the pulp actually appeared to hasten the death of the remaining pulp tissue. When infection of the remaining pulp is present in addition, the toxic effects of microorganisms added to those of cell breakdown products render recovery less likely. Thus, pulp capping, even under ideal circumstances, is a questionable procedure. Both Seelig[20] and Schroff[18] called it a "hit and miss" procedure. These experiments corroborate that viewpoint.

The formation of calcified tissue in exposed pulps was apparently stimulated initially by the presence of dentin chips which had been pushed into the pulp by the operation. These dentin fragments served as a stimulus for the deposition of a dentinoid tissue. There were indications of odontoblast-like cells lining the dentinoid material in some instances, especially where alkaline phosphatase was used, these cells were abundantly evident. Calcified tissue was produced even without the presence of drugs. The same observation had been made by Feldman,[5] Neuwirt,[10] Seelig,[20] Kreshover and Bevelander,[8] and many others.

At the end of 30 days it began to appear that, with the exception of calcium hydroxide, all of the calcium salts tested were injurious to dog pulp tissue. After 90 days, calcium hydroxide was the only calcium salt, of those tested, which was capable of maintaining the vitality of the remaining pulp tissue and of stimulating the formation of a dentin bridge. This was not merely a function of the pH of calcium hydroxide or the hydroxyl ion since pulp necrosis and periapical granulomas were caused by ammonium hydroxide used at the same pH as that of calcium hydroxide and calcium carbonate at a pH of 9.4.

Calcium hydroxide seems to supply several factors which are necessary for the rapid formation of reparative dentin. Among these are availability of the calcium ion at alkaline pH.[6] The other calcium salts tested were apparently unable to produce the same set of conditions. However, the possibility of dentin "bridging" without the use of drugs is not precluded. This was borne out by the finding of a deposition of reparative dentin where physiologic saline solution was applied to the pulp exposure (Fig. 7).

The formation of large amounts of reparative dentin with "bridging" of the exposed pulp has been regarded as indicative of favorable pulp response to injury.[1, 22-24] Whether or not this type of response is so favorable is now debatable. Reports, dealing with repaired calcium hydroxide-treated pulps, show that inflammation,[11] atrophy,[7, 9, 13] degeneration,[4, 12, 16, 17] and dentin resorption[2, 23] are possible sequelae, even in spite of dentin "bridging." Our experiments lead us to conclude, in agreement with Schroff,[19] that dentin "bridging" is a questionable criterion for pulp healing.

SUMMARY AND CONCLUSIONS

The pulps of 52 teeth of 3 dogs were exposed mechanically under aseptic conditions. In 50 per cent of the teeth, a blood clot was permitted to form before any further treatment was undertaken. In the other 50 per cent of the teeth, treatment was given immediately after pulp exposure. Various drugs were tested. Among these were calcium chloride, calcium carbonate, tricalcium phosphate, calcium hydroxide, ammonium hydroxide, alkaline phosphatase, and potassium penicillin. As controls, some pulps were treated with physiologic saline solution and some pulps were not medicated. The animals were sacrificed at 7-, 30- and 90-day intervals following operation. After decalcification, frozen sections of each tooth were made. These were stained with hematoxylin and eosin and studied microscopically. The radicular and apical regions of the teeth were studied in addition to the coronal. The following is a summary of the results together with some conclusions.

1. Calcium hydroxide was the only calcium salt, of those tested, which was capable of stimulating the pulp to lay down reparative dentin. However, the remaining pulp tissue was converted to granulation tissue after 90 days in one instance, remained normal in another.

2. Ammonium hydroxide, at the same alkalinity as calcium hydroxide, caused pulp necrosis in every instance.

3. Where an aqueous solution of 250,000 units of potassium penicillin was used, a necrotic pulp and apical granuloma was found in each instance.

4. The formation of a blood clot had no therapeutic effect on pulp repair on teeth of dogs.

5. The longer the interval of time elapsing between pulp exposure and death of the animal, the greater the likelihood of finding evidence of pulp damage.

6. A dentin "bridge" can take place even without the use of drugs. Dentin chips or fragments which are pushed into the pulp as a result of the operative procedure act as stimulants for the laying down of a dentinoid material.

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7. Pulp capping is a questionable procedure even under ideal circumstances.

8. The formation of a dentin "bridge" is a questionable criterion for successful pulp repair.

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THE REDUCING POWER OF HUMAN SALIVA AND ITS COMPONENT SECRETIONS

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HUMAN salivas reduce Fehling's solution and similar reagents for reducing sugars. On the assumption that the reducing material is D-glucose, the reducing sugar content has usually been found to fall between 10 and 35 mg. per 100 c.c. for salivas free of food materials. (For a summary of the past work, see Pigman and Reid,[1] and Lundqvist.[2]) The reducing power of the individual secretions composing human saliva has not been measured.

The nature, origin, and amount of the endogenous reducing substance in salivas are especially important since glucose, if present, would be expected to have an important influence on the nature and growth of the oral microorganisms.[3] Lundqvist[2] has provided evidence that true glucose, if present in human salivas, must have a concentration of less than 1 mg. per 100 c.c. despite the much higher apparent reducing power.

The present work was carried out with the purpose of determining the reducing power of the individual secretions which make up saliva. Methods for the collection of the secretions are now available.[4] Further evidence also was sought for the presence of "true" sugars in saliva and of the nature of the reducing substances.

EXPERIMENTAL

Saliva Collections.—For obtaining "stimulated" secretions, two procedures have been used in the present work: (a) chewing paraffin, and (b) swabbing the tongue once each minute with an applicator dipped in 1 per cent acetic acid. For the collection of whole saliva, the chewing of paraffin has been used as the only stimulation procedure, so that the saliva would not be contaminated by acetic acid. Only acetic acid has been used to stimulate the submaxillary and sublingual glands because chewing was difficult, if not impossible, with the collecting device in place. Parotid secretion was stimulated by both acetic acid and chewing paraffin.

For this study, whole saliva (the mixed fluid obtained directly from the human mouth) and the individual secretions of the major glands have been collected. The parotid gland secretion was collected by a suction cup. The secretions from the submaxillary and sublingual glands, uncontaminated by each other, were collected by means of a segregator.[4]

This work was supported by the Medical Research and Development Board, Office of the Surgeon General, Department of the Army (Contracts DA-49-007-MD-393 and 773) and by grants from the U. S. Public Health Service (USPH A-216, A-1303, and A-1225).

Received for publication Dec. 16, 1957.

Small amounts of saliva from the minor glands lining the oral cavity were obtained for some preliminary work. Small cellulose sponges were pressed against the oral mucosa with the flow from the major glands blocked by the appliances. After 30 to 60 minutes, the sponges were weighed and placed in a known volume of water.

Reducing Sugar Determinations.—The reducing power of whole saliva and of the individual salivary gland secretions was determined by a minor modification of the Folin-Malmros method.[5] No centrifugation or preliminary deproteinization was carried out unless indicated. Saliva, 0.2 to 0.4 c.c., was diluted with distilled water to 4 c.c. This diluted saliva was used in the place of the 1 to 1,000 dilution of protein-free blood. The Prussian blue color produced was measured by a Klett-Summerson photoelectric colorimeter with a filter having a maximum transmittance at 540 mμ.

Deproteinization Procedure.—The reducing power of whole saliva that had been deproteinized by the Somogyi method[6] was determined. For the determinations of the concentration of reducing substances left after deproteinization, equal amounts of saliva, 0.3 N barium hydroxide solution and 0.3 N zinc sulfate solution were added to varying amounts of water. The resulting mixtures were centrifuged. The reducing power of the supernatant liquids was measured by the Folin-Malmros micromethod.

This method of deproteinization was not found fully satisfactory for use with the Folin-Malmros method. For the low concentrations of the reducing substances in the deproteinized filtrates, standard solutions of glucose, treated with the deproteinization reagent, showed variations of ±100 per cent from the average values. Apparently zinc ions produced an interference.

Anthrone Determinations.—The carbohydrate content of whole and parotid saliva after deproteinization by the Somogyi procedure[6] was determined by the anthrone method as modified by Hanson, Schwartz, and Barker[7] and was calculated as apparent glucose. Deproteinization was carried out by the Somogyi method as above. The glucose solutions used as standard in the anthrone method were also carried through the Somogyi deproteinization procedure. To a portion of each saliva sample, the equivalent of 5.0 or 10.0 mg. per 100 c.c. of glucose was also added as a control. The recovery of the added glucose averaged 103 per cent for ten samples, with a range of 89 to 122 per cent.

Preparation of Lyophilized Salivary Materials.—The following human salivary materials were used: whole saliva with no external stimulation, parotid secretion produced during chewing of paraffin, submaxillary secretion produced without external stimulus. The saliva and individual secretions were led directly from the mouth into a 250 to 300 ml. round-bottomed flask cooled with acetone and solid carbon dioxide. Each flask contained no more than 20 to 40 c.c. The materials were lyophilized over a period of 6 to 12 hours. The dried products were scraped from the flasks, weighed, and stored in a desiccator at 0° C. until used. The range of dissolved total solids as gram per 100 c.c. for the salivary materials was: whole stimulated salivas, 0.6-1.0; parotid, 0.6-0.7; submaxillary, 0.3-0.5; sublingual, 1.2 Gm.)

Aqueous Ethanol Extraction of the Dried Salivary Materials.—Three types of procedures were used in attempts to extract sugars, if present, from the dried salivary materials. For each procedure, a known weight of dried material (about 0.1 Gm.) was weighed into a 12 c.c. conical centrifuge tube. A 1 to 2 c.c. portion of 50 per cent ethanol was added, and after the extraction period, the suspension was centrifuged. The supernatant solution was cooled or stored briefly at 0° C. Some of the extractions were made with hot 50 per cent ethanol and some with cold (2° C.) 50 per cent ethanol. For some of the extracts, additional material was precipitated by the addition of three volumes of absolute ethanol at 2° C.

Paper Chromatography Procedures.—The descending paper chromatographic procedure described by Partridge and Westall[8] was used for the analysis of the above extracts. The procedure was carried out at 25° C. using 6 by 18 inch strips of Whatman No. 1 paper in Pyrex jars with the trough assembly of the Research Equipment Corporation. After solutions had been spotted on the strips, the strips were exposed to the solvent vapors for at least 6 hours. The trough was then filled with a solvent consisting of the organic layer from a mixture of ethyl acetate-acetic acid and water (3:1:3, by volume). The solvent was allowed to flow for about 6 hours, until it had reached within about 1 inch of the end of the strip. The glucose spots moved approximately 3 cm. during this period. (When the solvent was kept for a week, the rate of movement doubled.)

The strips were air dried for 18 hours at room temperature, sprayed with indicating reagent and, when necessary, dried in an oven at 110° to 125° C.

The following spray reagents were used: 3,5-dinitrosalicylic acid,[9] aniline hydrogen phthalate,[10] aniline hydrogen oxalate,[11] aniline phosphate,[12] and aniline acetate. The aniline acetate was a new reagent prepared similarly to the aniline hydrogen oxalate. The published directions for the aniline phosphate reagent were found to be incomplete. The reagent was made by mixing one volume of 0.2 M solution of aniline in *n*-butanol with two volumes of a 0.2 N solution of phosphoric acid in *n*-butanol. The original butanol was saturated with water at room temperature. The literature[12] prescribed 2 N solutions.

A preliminary survey of the sensitivity of these reagents was made by spotting known concentrations of D-glucose on the paper, drying the spots, and spraying according to the recommended procedures. The amount of D-glucose in the spots varied systematically over the range of 0.25 to 4.0 μg, with the spot diameters varying between 5 and 10 mm.

With the aniline hydrogen phthalate and aniline phosphate reagents, 0.5 μg of D-glucose in a spot of 10 mm. diameter could be detected. By examination with short-wave ultraviolet light, the sensitivity was somewhat improved and 0.4 μg was clearly evident. When the strips were kept for 1 month, the spots with even less glucose became clearly visible. When the aniline phosphate reagent was made up in water instead of butanol, the sensitivity was

less (1 μg) and considerable diffusion of the spots occurred. The sensitivity of the other reagents was about as follows: aniline acetate, 1.5 μg; aniline oxalate, 1 μg; 3,5-dinitrosalicylic acid, 0.75 μg.

The sensitivity of the aniline phosphate and aniline phthalate reagents to glucose was then tested on an actual chromatogram using the descending technique described above. The amount of glucose in the original spots varied over the range 0.5 to 10 μg. A minimum of about 1 μg of glucose on a final spot about 8 mm. in diameter was detectable; 0.5 μg was barely detectable with the short-wave ultraviolet light.

Analysis of the Extracts of the Dried Salivary Materials.—A 0.025 to 0.050 c.c. portion of the extracts was immediately spotted on a strip of Whatman No. 1 filter paper and subjected to the paper chromatography procedure described above. On the same strips 2 μg of glucose or 6 μg of glucosamine or *N*-acetylglucosamine were spotted as controls. Glucose and *N*-acetylglucosamine were always clearly distinguishable after the chromatographic procedure, but glucosamine was not always detectable. The descending method was used, and the solvent was the organic layer from a mixture of ethyl acetate, acetic acid, and water (3:1:3). For identification of reducing sugars, the aniline hydrogen phthalate spray reagent was used. For the detection of glucosamine and *N*-acetylglucosamine, the hexosamine reagent of Partridge[8] was used. The ninhydrin reagent (I) of Block[13] was also tested.

Reducing substances were determined on the extracts by the use of the Folin-Malmros method. In later work the anthrone method was used to determine the carbohydrate content of some of these extracts.

RESULTS

Relative Reducing Properties of the Individual Salivary Secretions.—Human whole saliva and all of the major salivary gland secretions were shown in these studies to contain materials which exhibit reducing power to the Folin-Malmros ferricyanide reagent. This represents the first study of the reducing materials in the individual salivary gland secretions.

Table I gives a summary of the reducing power, calculated as milligram of apparent glucose, in human whole saliva and the salivary gland secretions. Table II compares the reducing powers of each secretion from 3 subjects.

The reducing power of the stimulated whole salivas did not appear to differ appreciably from the reducing power of the resting whole saliva. No significant difference could be observed between the resting and stimulated submaxillary secretions. The average reducing power of the stimulated secretion from the parotid gland was less than that of the resting parotid gland secretion, and was significant at $p<0.001$.

Both the parotid and submaxillary secretions often appear to contain less reducing material than whole saliva. This is generally true of the over-all averages and also, with one exception, of the averages for single individuals. If this difference is real, the additional reducing materials may come from the salivary precipitate in whole saliva, the secretion of the minor glands and that

of the sublingual glands. The secretions of both the minor glands and the sublingual glands appear to have a higher concentration of reducing substance than the secretions of the other glands, but the number analyzed was small.

TABLE I
SUMMARY OF REDUCING POWER (AS GLUCOSE) OF HUMAN SALIVAS AND SECRETIONS

SALIVA TYPE	STIMULATION*	NO. OF DETERMINATIONS (AND INDIVIDUALS)	RANGE (MG./100 C.C.)	AVERAGE (± S.D.) (MG./100 C.C.)
Whole	Stim. (CP)	24 (7)	9.0-25.6	14.5 ± 3.5
Whole	Resting	28 (7	7.2-31.5	13.3 ± 5.7
Submaxillary	Stim. (AA)	7 (3)	5.2- 9.2	6.7 ± 1.6
Submaxillary	Resting	17 (7)	4.4-13.6	7.2 ± 1.7
Parotid	Stim. (CP, AA)	14 (6)	3.8-15.5	6.8 ± 2.4†
Parotid	Resting	15 (7)	5.1-27.6	12.7 ± 6.4†
Sublingual	Stim. (AA)	4 (2)	14.7-27.0	19.0 ± 4.8
Sublingual	Resting	2 (2)	7.0-30.0	18.5
Accessory	Resting	3 (3)	18.8-41.4	27.4

*Methods of stimulation: Stim. = stimulated by; CP = chewing paraffin; AA = 1% acetic acid swabbed on tongue; Resting = no direct stimulation.
†$P < .001$ for difference between resting and stimulation.

TABLE II
THE DISTRIBUTION OF REDUCING POWER (AS GLUCOSE) IN THE SALIVARY SECRETIONS FROM 3 SUBJECTS

SALIVARY SECRETION	STIMULUS*	NO. OF DETERMINATIONS	RANGE (MG./100 C.C.)	AVERAGE (MG./100 C.C.)
		Subject J. M.		
Whole	None	5	12.2-31.5	21.5
Whole	CP	2	18.0-25.6	21.8
Submaxillary	None	3	8.8-13.6	10.8
Submaxillary	AA	3	5.2- 9.2	7.8
Parotid	None	3	8.6-15.3	13.0
Parotid	AA	3	5.9-10.6	7.9
Sublingual	None	1		7.0
Sublingual	AA	3	14.7-27.0	19.9
Minor	Sponges	1		41.4
		Subject J. R.		
Whole	None	13	7.2-14.1	10.3
Whole	CP	10	9.0-17.4	13.1
Submaxillary	None	7	4.5- 7.4	6.0
Submaxillary	AA	2	5.2- 6.1	5.6
Parotid	None	3	20.1-27.6	23.4
Parotid	CP	6	4.2- 5.8	4.7
Parotid	AA	1		15.5
		Subject R. N.		
Whole	None	4	16.2-19.6	17.4
Submaxillary	None	1		6.2
Submaxillary	AA	1		7.2
Parotid	None	3	7.4-18.0	10.9
Parotid	AA	1		11.7
Sublingual	None	1		30.0
Sublingual	AA	1		16.2
Minor	Sponges	1		18.8

*The methods of stimulation are abbreviated as given in Table I. Sponges = placing cellulose sponges over the minor glands.

Whole saliva, especially when stimulated by chewing, contains considerable amounts of precipitated material which can be removed by centrifugation. Since the significance of this material in salivary analysis is unknown, the values reported in the present work were for uncentrifuged salivas. In several instances, the effect of centrifugation was determined. The centrifuged salivas

showed only 65 to 80 per cent of the reducing power of the uncentrifuged solutions. It should be noted, however, that the individual secretions were generally clear, and free of suspended material.

Change of Reducing Power With Time.—The increase of reducing power of whole saliva upon standing was measured. Unstimulated whole saliva was collected in an ice bath and several drops of toluene were added. Immediately after the first portion was withdrawn, the sample was placed in a water bath at 32° C. The increase of reducing power with time is shown in Table III.

TABLE III

INCREASE OF REDUCING POWER (MG. PER 100 C.C.) OF WHOLE SALIVA WITH TIME AT 32° C.

	TIME (HR.)					
	0	0.75	2	3	4.5	5
Sample I	7.6		11.8	11.7	11.8	
Sample II	10.7	12.4		12.2		13.4

Because the reducing power of saliva increased upon standing at 32° C., analyses generally were made on samples collected in an ice bath, and analyzed within a few hours.

Reducing Power of Deproteinized Salivas.—Two samples of whole saliva were deproteinized by the Somogyi procedure.[6] The filtrates showed reducing power corresponding to about 1 mg. per 100 c.c. of D-glucose, if present. When the same procedure was carried out on a saliva sample to which 20 mg. of glucose per 100 c.c. had been added, the recovery was 98 to 102 per cent. Since the Somogyi reagents seem to interfere with the Folin-Malmros method, especially for low concentrations of reducing sugar, these results have only semiquantitative significance.

The anthrone method for saccharides gave more dependable results on the deproteinized whole salivas and parotid secretions. Eight samples of whole stimulated salivas from 4 individuals showed a range of apparent "glucose" of 1.5 to 4.5, average 2.6 mg. per 100 c.c. Three samples of parotid secretion, averaged 1.0 mg. per 100 c.c. of apparent "glucose," range 0.8 to 1.2.

Reducing Power of Alcoholic Extracts of Lyophilized Salivary Materials.—Since D-glucose and other monosaccharides should be somewhat soluble in aqueous alcohol, lyophilized whole salivas and parotid and submaxillary secretions were extracted with hot 50 per cent ethanol, and the reducing power of the extracts was determined. The concentration of reducing substance in the extracts was determined as glucose by the Folin-Malmros method and calculated to the basis of 100 c.c. of original saliva or secretion.

For whole saliva, the reducing material extracted by hot (50 per cent) alcohol extracts contained several substances which showed reducing power. When this extract was frozen and thawed, material precipitated. The precipitate contained reducing material since the reducing power of the solution decreased from 5.1 mg. to 3.8 mg. per 100 c.c. Extractions were then carried out at 0° C., and the extracts were centrifuged at room temperature. These extracts

from whole saliva, parotid and submaxillary solids contained reducing substances corresponding to 2.1 to 4.5 mg. per 100 c.c., calculated as glucose on the basis of the original saliva. The anthrone method gave a somewhat higher result for the carbohydrate calculated as glucose in similar extracts of dried whole saliva; the apparent "glucose" concentration was 6.2 mg. per 100 c.c., and this was reduced to 5.6 mg. per 100 c.c. by deproteinization by the Somogyi procedure. Since recovery experiments with dried salivas which contained known amounts of glucose (5 or 10 mg. per 100 c.c. in the original saliva) gave recoveries of only 50 to 60 per cent under these conditions, the above values probably represent about half the concentrations of the ethanol (50 per cent) soluble material in dried salivas.

To remove still more constituents under conditions such that sugars in small amounts should still be soluble, a third set of extractions were carried out in the cold, and cold ethanol was added to a final concentration of 87 per cent. A white gelatinous precipitate was separated by cold centrifugation. The resulting 87 per cent ethanol solutions showed only 0.5 to 0.8 mg. per 100 c.c. of reducing power for the solids from whole saliva, parotid, and submaxillary secretions by the Folin-Malmros method. The anthrone method showed 0.6 mg. per 100 c.c. for a similar extract of whole saliva. Similar experiments on a saliva containing known amounts of added D-glucose indicated a recovery of about 40 per cent under these conditions.

Reducing Properties of Human Mucin and Parotid Protein.—The average reducing power of the two samples of crude mucin prepared according to the method of Knox and Still[14] was 2.9 per cent calculated as glucose by the Folin-Malmros method. A protein isolated from cattle parotid glands by Platt[15] had a reducing power of 2.5 per cent of its weight of glucose. No preliminary acid hydrolyses of these products were made before analysis.

Use of Paper Chromatography to Identify Sugars in Salivary Secretions.—The concentrations of sugar indicated by the reducing sugar methods are too small to make possible the direct paper chromatography of salivary secretions, especially as it became apparent that a variety of materials are responsible for the reduction. In order to increase the sensitivity of the identification, the secretions were concentrated by lyophilization and extracts made in 50 and 87 per cent ethanol. Aliquots of these were used for analyses by paper chromatography using known concentrations of D-glucose, D-glucosamine and *N*-acetyl-D-glucosamine as standards and using spray reagents of known sensitivity (see experimental section).

No evidence for the presence of sugars or hexosamines could be obtained under conditions such that 0.3 mg. of glucose or 1 to 3 mg. per 100 c.c. of glucosamine or *N*-acetylglucosamine would have been detected. These upper limits were determined by experiments in which known amounts of D-glucose (5 or 10 mg. per 100 c.c.) were added to a whole saliva which was then treated in the same manner as those for the paper chromatographic work; an aliquot of the same saliva sample (without added glucose) was studied similarly. "Glucose" determinations by the anthrone method on the extracts made from the

salivas with known amounts of added D-glucose, indicated recoveries of 50 to 60 per cent in the ethanol (50 per cent) extracts. The maximum concentration of 0.3 mg. per 100 c.c., set for glucose, implies a 50 per cent recovery.

Although no spots corresponding to known sugars could be found, the chromatograms of the extracts of lyophilized salivary material with hot and cold 50 per cent ethanol showed a long streak of material which fluoresced under ultraviolet light before development with the spray reagents. It was not developed by the aniline phthalate reagent, but apparently showed up with the ninhydrin spray as the same color as was given by glucosamine. The hexosamine spray showed the presence of a bright yellow streak developed from the 50 per cent alcohol spots, and a yellow spot with an R_f of about 0.5 for the extracts from the cold 50 and 87 per cent ethanol extracts of each type of secretion. The yellow color was developed by the ordinary procedure for hexosamines and also by treatment with *p*-dimethylaminobenzaldehyde without prior treatment with acetylacetone or base. Similar materials have been reported in human urines.[16]

DISCUSSION

Each of the known component secretions of human saliva contain substances which reduce the Folin-Malmros reagent, but no evidence could be found for the presence of simple sugars or hexosamines.

The heterogeneous nature of the reducing materials of saliva has been indicated earlier by a study of a large concentrated sample of pooled saliva.[17] Because of the probable changes which occurred during the preparation of the sample, the values for this material have only qualitative significance. Dialyzable reducing material, nondialyzable reducing material, and nondialyzable, nonreducing carbohydrates were found. The total carbohydrate by the anthrone method was 70 per cent greater than the reducing sugar (10 mg. per 100 c.c. as glucose). Not more than 2 mg. per 100 c.c. of fermentable sugar was present, but this fraction might have been lost in the preparation of the sample.

Evidently the reducing substances of salivas are highly heterogeneous. Part of the reducing material is present in the precipitate in stimulated whole saliva. The materials forming mucin seem to be a source of some of the reducing power. Since mucin (or its component materials) seems to be a major "component" of salivas and submaxillary secretions, it may provide much of the reducing properties of these secretions, as pointed out earlier by Gore.[18]

Some of the reducing power exhibited by whole saliva seems to arise from materials elaborated by the parotid gland, which in man is generally considered to be a serous gland. The parotid gland secretion contained appreciable reducing power, some of it probably from a protein component.

Several independent methods indicate that the amount of true glucose in saliva and its component secretions is very low, if any is present at all. This finding confirms that of Lundqvist.[2] No evidence for any monosaccharides, oligosaccharides, or hexosamines could be obtained. In terms of the evident heterogeneity of the salivary reducing substance, the values given in Tables I and II have no absolute significance as glucose or "reducing substance" in salivary secretions, but have their value only for relative comparisons.

SUMMARY

The reducing power of whole salivas and of parotid, submaxillary, sublingual, and mucosal secretions has been measured by the Folin-Malmros method and reported as apparent glucose. Generally, the parotid and submaxillary secretions have less reducing power than the sublingual and mucosal secretions. The only noticeable effect of stimulation was observed for the parotid gland, which showed a decreased reducing power upon stimulation.

In agreement with the earlier results of Lundqvist, no evidence was found for the presence of any free sugars, although several different methods were tried. The various methods indicate a probable maximum concentration of about 0.5 mg. per 100 c.c.

The reducing substances of the salivary secretions seem to be a heterogeneous mixture of various carbohydrates, proteins, glycoproteins and some small molecular weight materials.

The sensitivity of a number of spray reagents useful for the detection of sugars and hexosamines on paper chromatograms is reported.

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A BACTERIOLOGIC CENSUS OF HUMAN SALIVA

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ALTHOUGH qualitative studies have described many genera and species from numerous oral locations, only scant information is available as to the relative populations of even those organisms which can be cultured. The present study was initiated to obtain such information.

Kraus and Gaston[1] presented the population means for total aerobes, total streptococci, and *Streptococcus salivarius*. Krasse[2] determined the means for streptococci in addition to those of lactobacilli and yeast. Green and Dodd[3] compared 7 salivary populations of caries-immune and caries-susceptible subjects. A limited census of salivary organisms has on occasion been determined as an incidental part of an investigation.[4, 5]

The framework of any census is dependent upon the availability and excellence of the selective media. Recently, several improved selective media have been described. For example, Rogosa, Mitchell, and Wiseman[6] devised a medium for lactobacilli, Douglas[7] and Rogosa[8] used lactate-containing media to demonstrate the large population of Veillonella present in saliva, and an improved selective medium for Fusobacterium has been introduced by Omata and Disraely.[9]

Several quantitative studies have been made on saliva in which 1 to 3 groups of organisms have been surveyed. Experimental designs have been markedly heterogeneous, especially as to saliva analyzed (stimulated or nonstimulated), agitation of specimen (none or mechanically shaken), and media. In only a few studies[3, 10, 11] have as many as 7 to 10 salivary bacterial populations been investigated.

Thus, a study which surveyed a large number of salivary bacterial categories simultaneously, using a simple and adequately described experimental design, seemed desirable. This study constitutes a census of 14 groups of organisms cultured from human saliva.

METHODS

Saliva Specimens.—Each of 14 subjects contributed saliva specimens on 10 separate occasions, the 10 specimens for any one person being collected over a 5-month period. Subjects ranged between 25 and 40 years of age (10 males and 4 females) and were departmental staff personnel and graduate students.

Aided in part by a grant from the College of Medicine Fund of the State University of Iowa.
Received for publication Dec. 20, 1957.

Specimens were collected between 9:15 and 10:15 A.M., the subjects spitting 4 to 6 ml., of saliva (not specially stimulated) into sterile cotton-plugged test tubes. Contributors were cautioned to avoid contamination of the specimen during collection. Samples were not collected within 1 hour following the drinking of coffee, eating of breakfast, or from a subject receiving antibiotics.

Serial Dilutions.—Tenfold serial dilutions of the saliva samples were prepared in 9.0 ml. sterile saline. Just prior to making the dilutions, saliva specimens were drawn up and down in a 1.0 ml. pipette 10 to 15 times. A transfer of 1.0 ml. of saliva was made to the first dilution tube, and the specimen emptied on the side of the test tube, 1 to 2 cm. above the saline. A different sterile pipette was used for mixing and transferring to the second dilution tube. This procedure for mixing and transferring was continued through 7 tubes, employing a different pipette for each tube. To maintain consistency in the preparation of the serial dilutions, the same investigator handled this procedure throughout the study.

Plating, Incubation, and Counting.—All media were inoculated within 1 hour following collection of the specimens.

Spread plates were prepared for enumeration of total streptococci, starch hydrolyzers, Neisseria, Fusobacterium, Leptotrichia, salt-tolerant micrococci, Candida, and coliform organisms. Pour plates were employed for enumeration of total aerobes, total anaerobes, *Streptococcus salivarius,* Veillonella, lactobacilli, and hydrogen sulfide-producing organisms. To ensure countable plates, two appropriate dilutions were selected for inoculation on each of the various media. A satisfactory range of dilutions had been determined in preliminary trials. Duplicate platings were made for all dilutions used. The inoculum for spread plates was 0.1 ml. and for pour plates was 1.0 ml. of the selected dilution.

For growth of total anaerobes, Veillonella, lactobacilli, Fusobacterium, Leptotrichia, and hydrogen sulfide-producing organisms, plates were incubated anaerobically in glass desiccator jars. Anaerobiosis was obtained by twice evacuating these jars to 600 mm. Hg and filling with CO_2. After a third evacuation, nitrogen gas was introduced to provide an environment of 90 per cent N_2 and 10 per cent CO_2. All other media were incubated aerobically. All cultures were incubated at 37° C. for 4 days before counting.

Colony counts were performed in natural daylight without magnification aids and with both investigators participating. Plates which contained 10 to 70 colonies were selected for counting. For the enumeration of starch hydrolyzers, however, plates having fewer than 10 colonies showing hydrolysis were used for counting.

Microbial Categories Surveyed and Media Employed.—

Total aerobes and total anaerobes: The medium used for both categories was 5 per cent sheep blood in sterile trypticase soy agar (BBL).

Total streptococci: The medium employed by Kraus and Gaston[1] in their determination of this group was modified as follows: sheep blood and trypticase soy agar were substituted for horse blood and brain heart infusion agar.

Streptococcus salivarius: Niven's selective medium[12] was used; the large mucoid colonies were counted.

Neisseria: Growth of members of this genus was obtained on 3 per cent sheep blood in trypticase soy agar. Colonies which turned black following flooding with freshly prepared 1 per cent aqueous solution of N, N-dimethyl-p-phenylenediamine monohydrochloride (Eastman) were counted as Neisseria. Identification of representative black colonies verified the acceptability of this procedure.

Veillonella: The medium-sized discus-shaped colonies which grew on Rogosa's selective medium[8] were counted.

Lactobacillus: The lactobacillus selective medium (BBL) formulated by Rogosa, Mitchell, and Wiseman[6] was used for growth of members of this genus.

Fusobacterium: The medium and procedure suggested by Omata and Disraely[9] were modified as follows: (1) spread plates were used since we felt that the characteristic surface colonies of fusobacteria were more reliably recognized than deep colonies, (2) with the recommended medium a high proportion of the growth was identified as micrococci; the majority of these were eliminated by increasing the crystal violet concentration from 10 mg. per liter to 20 mg. per liter, and (3) to enrich the basal medium 5 per cent sterile sheep serum was used instead of horse serum, ascitic fluid, or starch.

Leptotrichia: The flat Medusa head-like colonies present on the medium used for Fusobacterium were tentatively identified and counted as Leptotrichia.

Candida: Colony counts of this genus were made on mycological agar (Difco) which contained penicillin (50 units per ml.) and streptomycin (50 μg per ml.).

Salt-Tolerant Micrococci: Chapman-Stone micrococcus medium (BBL) was utilized for growth of these organisms.

Starch Hydrolyzers: For the preparation of the medium for growth of these organisms, 10 Gm. of soluble starch (Merck) were suspended in 500 ml. distilled water and added to 500 ml. of heated trypticase soy agar (40 Gm. in 500 ml.). Colonies exhibiting hydrolytic activity were recognized by the clear zone surrounding the colony after flooding the medium with Gram's iodine solution.

Hydrogen Sulfide-Producing Organisms: This medium contained 36 Gm. peptone iron agar (Difco) and 30 Gm. cystine trypticase soy agar (BBL) per liter of medium. Black colonies on this medium were counted as H_2S producers. Slightly higher counts were obtained if the solidified pour plates were layered with this medium prior to anaerobic incubation.

Sterilization of Medium.—To prevent contaminating growth during storage, the salivarius medium and Veillonella medium were autoclaved the day of preparation. All other media were heated to boiling during preparation, dispensed in clean flasks, and stored at 4° C. Just prior to use, all media were autoclaved. Medium stored longer than 10 days was rarely used.

Dental Examination.—The mouth of each subject was inspected for plaque accumulations, location and extent of calculi, presence and severity of gingival lesions, and number of decayed, filled, and missing teeth.

Statistical Analysis.—Throughout the investigation the average of duplicate plate counts was recognized as the count for that category, and calculations were made on these average counts. For each individual the arithmetic average of the 10 counts for each category was determined; the standard deviation of these means was calculated using the formula[13]

$$s = \sqrt{\frac{\Sigma X^2}{N} - \left(\frac{\Sigma X}{N}\right)^2}.$$

Similarly, the mean and standard deviation for each category were calculated.

The "t" test[13] was employed for the determination of those subjects who exhibited statistically significant high or low means as compared to the category mean. All categories were analyzed, and the 1 per cent level of confidence was recognized as statistically significant.

RESULTS

The group means, standard deviations, and range of the subject means for each category are presented in Table I. According to their means the categories have been placed into high ($n \times 10^6$ per ml. of saliva), intermediate ($n \times 10^3$), or low ($n \times 10^2$) population divisions.

TABLE I

A CENSUS OF 14 BACTERIAL CATEGORIES IN SALIVA*

	CATEGORY	MEAN OF 14 SUBJECTS	STANDARD DEVIATION	RANGE OF SUBJECT MEANS
High count categories ($n \times 10^6$ per ml.)	Total anaerobes	110	112	10 -384
	Total aerobes	40	34	5 -114
	Total streptococci	18	15	2 - 57
	Veillonella	17	15	2 - 49
	S. salivarius	11	11	0.6 - 36
	Starch hydrolyzers	5	3	1 - 11
	H_2S producers	2	1	0.3 - 4
	Neisseria	2	2	0.08- 7
Intermediate count categories ($n \times 10^3$ per ml.)	Fusobacterium	56	45	7 -192
	Lactobacillus	35	64	0 -252
	Salt-tolerant micrococci	5	11	0.01- 42
	Leptotrichia	3	6	0 - 25
Low count categories ($n \times 10^2$ per ml.)	Candida	2	3	0 - 6
	Coliforms	1	4	0 - 15

*The salivas of 14 subjects were analyzed bacteriologically on 10 separate occasions for each of the categories. The mean of the 10 counts for each category was determined for all subjects. Each category mean was obtained by arithmetic average of the 14 subject means.

The count for any one subject in any category showed a considerable fluctuation (± 100 per cent of mean) from examination to examination. Each subject had a characteristic zone about which the counts would fluctuate; e.g., subjects with high counts fluctuated about their respective high means, while

subjects with low counts fluctuated around low means. If a specimen from a subject showed an increase or a decrease in one category, a parallel change was seen in other categories. In all categories there was a wide range of subject means.

In this study, the group mean anaerobe census was approximately 3 times the aerobe population. The 40 million total aerobe census compares favorably with the 41 million obtained by averaging the 4 control aerobe groups of Bender,[4] and is within an acceptable range of the mean total aerobe counts obtained by Kraus and Gaston.[1] Perhaps coincidentally, the mean total aerobe population for the 4 females of this study was practically identical to that obtained for the female group of Kraus and Gaston.

The total streptococci, with a group mean of 18 million per milliliter of saliva, represented 45 per cent of the total aerobes; these findings thus confirm those of Kraus and Gaston[1] who obtained a 42 per cent value. Since the total streptococcus count was made on plates incubated aerobically, the strictly anaerobic streptococci are not included in this census.

Streptococcus salivarius comprised 61 per cent of the total streptococcus population. Kraus and Gaston[1] reported this organism to represent 50 per cent, and Krasse[14] 49 per cent, of the total aerobic streptococci. Obviously, this organism is a major component of the oral streptococcus population.

The Veillonella mean population for the group was 17 million per milliliter of saliva. This category represented 17 per cent of the total anaerobes, and together with the total streptococci, a facultative group, accounted for 35 per cent of the total anaerobe mean population. Rogosa,[8] using the same medium, found the mean Veillonella population of 145 subjects to be 35 million per milliliter of saliva. However, in his study, stimulated saliva specimens were agitated for 2 minutes by machine. Such treatment could separate small groups of individual cocci from clusters, and might easily account for the discrepancy of the counts.

Since the Veillonella seem to utilize lactate better than most other oral organisms, it would appear reasonable to speculate that in mouths where lactate is plentiful (high lactobacillus counts) the Veillonella population would be high. The information obtained in this study cannot support or reject this hypothesis. Subjects with low Veillonella counts (1 to 3 million per ml. of saliva) had less than 1,000 lactobacilli per milliliter of saliva and were relatively immune to dental caries. These subjects, however, exhibited correspondingly low counts for most categories. Subjects with moderately high Veillonella counts (greater than 20 million per ml.) exhibited moderately high lactobacillus counts (greater than 20 thousand per ml.) and had a greater past caries activity than subjects with low counts. One subject with a significantly high lactobacillus count had a Veillonella count just under the group mean of 17 million per milliliter of saliva.

The starch hydrolyzers, with a group mean of 5 million per milliliter of saliva, represented 12 per cent of the total aerobe population. The major starch hydrolyzers were alpha hemolytic streptococci. Since *S. salivarius* is nonhemolytic and does not hydrolyze starch, it is probable that the mean starch-hydrolyzer counts represent an additional group of streptococci, possibly *S. bovis* and, thus, account for a further portion of the total aerobe and total streptococcus populations. The medium used for this census was not entirely satisfactory. Counts of duplicate plates varied more than was desired, possibly because more than 10 colonies of hydrolyzers per plate obscured the results. There was no correlation between past or present caries activity and starch-hydrolyzer counts.

The mean of hydrogen sulfide-producers for the group was 2 million per milliliter of saliva. The ratio of hydrogen sulfide-producing colonies to non-H_2S-producing colonies was about 1 to 20. Two major types of black colonies were encountered: (1) tiny pin-point colonies, and (2) large snowball type. Streptococci and fusobacteria were isolated from both colony types. Subjects with gingivitis and calculus formation had high counts.

The group mean for Neisseria was 2 million per milliliter, 5 per cent of the total aerobe mean. *N. catarrhalis* and *N. pharyngis* were the species most commonly encountered. The demand of this genus for an aerobic atmosphere might lead one to speculate that subjects with clean mouths would have more aerobes, hence high Neisseria counts. Actually, the reverse was true. Subjects with high Neisseria counts had gingivitis and calculus deposits, whereas low Neisseria counts were found in subjects with slight, or no, gingival pathosis and little, if any, calculus. The range of means for the Neisseria was greater than that of most categories, and it is felt that further investigation might be worthwhile in elucidating the meaning of the wide range.

The Fusobacterium mean of 56,000 per milliliter of saliva obtained in this study is in reasonable agreement with the counts of Slanetz and Reynolds[5] and those of Omata and Disraely[9] when they employed the potato extract medium of Slanetz and Rettger.[15] However, Omata and Disraely, using their recently developed selective medium, obtained Fusobacterium counts on the order of 800,000 per milliliter of saliva. In the pour plate method used by Omata and Disraely, it is possible that organisms other than fusobacteria were counted as Fusobacterium. The occurrence of Fusobacterium as a major group on our H_2S medium infers the presence of a higher fusobacteria population than was obtained on the Fusobacterium medium. If this population were estimated as one third of the H_2S producers, then the census would approach that reported by Omata and Disraely.

It should be emphasized that the group mean micrococcus count is a census of salt-tolerant micrococci. *Micrococcus pyogenes* var. *albus* and *M. pyogenes* var. *aureus* were isolated from 10 of the 14 subjects; only one subject consistently had a high *M. aureus* population, and this subject exhibited advanced gingivitis. The carrier state fluctuated for other subjects.

An estimated total micrococcus population in the low millions is proposed on the basis that micrococci were encountered on the Neisseria plates in approximately the same numbers as Neisseria. Using this estimate, it would appear that streptococci outnumber the micrococci approximately 9 to 1 and that micrococci would account for 5 per cent of the total aerobe population. This estimate compares favorably with the values obtained by Green and Dodd.[3]

In this study, the subjects who contributed saliva were for the most part past the age where active dental caries is encountered. It is not surprising that lactobacillus counts for the group were low. One subject, the youngest, had open carious lesions and a lactobacillus count significantly higher than the group mean.

On the surface of the Fusobacterium medium, flat, translucent Medusa head-like colonies, 4 to 6 mm. in diameter, were cultured from 12 of the 14 subjects (Fig. 1, *A*). These colonies resembled those characterized by other investigators as Leptotrichia.[16-18] The organisms comprising these unusual colonies (Fig. 1, *B*) were filamentous, straw-shaped, nonbranching, gram-positive rods (20 to 200 μ) and satisfied the morphologic characteristics of the poorly defined Leptotrichia. At times, one end was pointed, and occasionally a helix could be found along the length of the rod. These organisms were tentatively identified as Leptotrichia. Subjects with clean mouths had lower Leptotrichia counts than subjects with gingivitis and tartar formation.

Candida, usually *C. albicans,* were isolated from 4 of the 14 subjects. Subjects were either Candida positive or negative; no particular dental condition could be associated with the presence or absence of Candida.

Coliform organisms (*Alcaligenes* and *Aerobacter* species) were encountered less frequently than Candida, and only one subject consistently carried these organisms. Coliforms were isolated from each of 3 other subjects 2 to 3 times. It was of interest that the subject who consistently carried the coliforms was engaged to a girl who also served as a subject but from whom coliforms were not isolated over a period of several months. Using the method employed for the qualitative bacteriologic analysis of water, Nolte and Marston[19] isolated coliform organisms from 32 per cent of a large group of subjects. The large inoculum and the liquid media used by these workers could account for the higher incidence. They found no correlation between the presence of aerogenes types and caries activity and inactivity.

True vegetarians are uncommon in this country and the opportunity to examine the salivary flora of a few vegetarians was considered fortunate. Three graduate students from India who ate no meat, fish, or fowl cooperated in this study. Saliva from these vegetarians was studied as outlined for the group of 14 non-vegetarians. No statistical differences were found between the vegetarians and non-vegetarians for any of the 14 categories surveyed (neither would a statistical difference have been found had a 5 per cent level been used). One additional observation concerning diet and oral flora deserves mention. A subject from whom coliforms were never isolated during this

study boarded for 2 years with the 1 subject who consistently carried the coliforms in relatively high numbers.

Population counts were considered in relation to the speed of saliva collection. Some contributors had no difficulty in collecting a saliva sample; however, a few required 15 to 30 minutes for the collection. Fast collectors

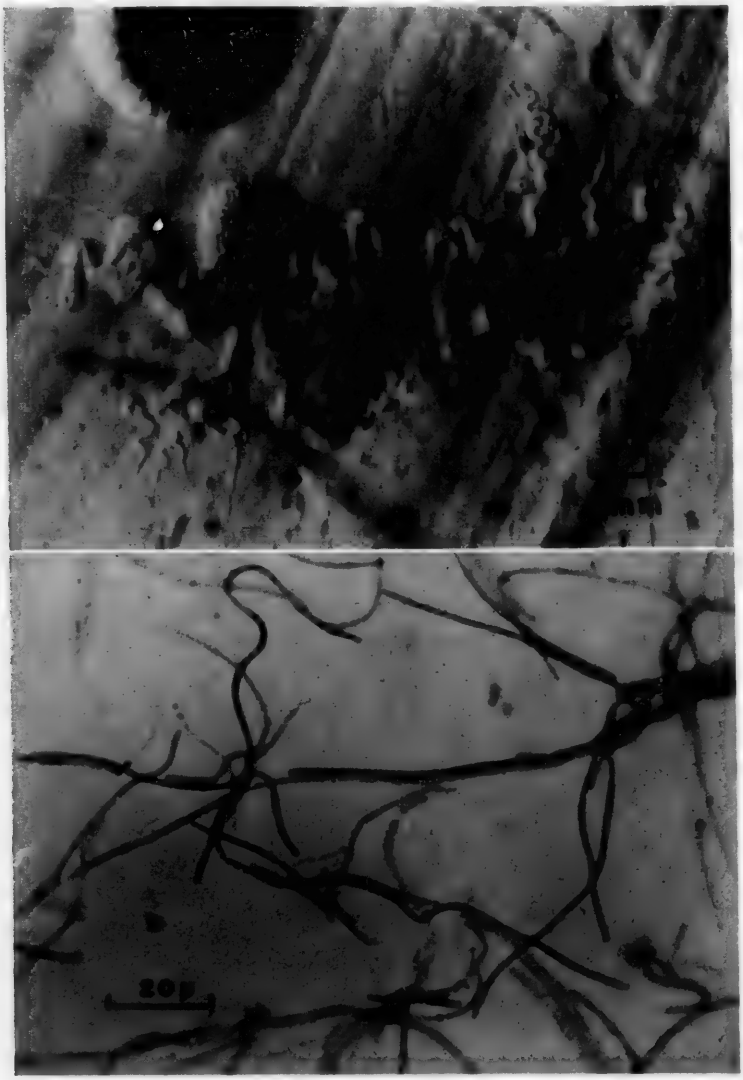

Fig. 1.—*A*, A Leptotrichia colony on Fusobacterium medium showing characteristic irregularity and Medusa-head design. Note the numerous tentacle-like projections extending from the edge of the colony. The two round colonies with entire edge are fusobacteria. The plate had been inoculated with saliva and incubated anaerobically at 37° C. for 4 days.

B, Gram stain of a similar Leptotrichia colony on the same plate.

definitely had higher counts than slow collectors. A statistical analysis of several categories failed to show that the difference in counts between the groups could not have been due to chance alone.

While it may not be representative of other groups, the females of our group exhibited lower counts than the males. For several categories the differences were significant at the 5 per cent level of confidence but not at 1 per cent.

In this study, subjects who exhibited low counts for most categories were nonsmokers or occasional smokers, and subjects having high counts were smokers.

Table II lists subjects with statistically high or low counts as compared to the category mean and brief descriptions of the oral condition of each subject. Five categories (Neisseria, Micrococcus, Fusobacterium, Leptotrichia, and hydrogen sulfide-producers) seemed to constitute an "index of gingival health." Those subjects (I, II, III, IV, V, XII, XIV) who had statistically significant low counts for one or more of the 5 categories had healthy gingiva and little or no calculus. Alternatively, subjects (VIII, IX, X, XI, XIII) who exhibited significant high counts in one or more of the 5 categories had varying degrees of gingivitis and calculus deposits. Subject IX who was high in 4 of the 5 categories was in need of immediate periodontal treatment. This same subject had counts near the group mean in the remaining categories, indicating that not all bacterial populations are unusually high in periodontal disease. Subject XIV is of interest in that he had a significantly low Leptotrichia count but exhibited early periodontosis disease. Subjects IX and X exhibited unusually high populations in a number of categories. Both subjects had reasonably healthy mouths, although it was apparent that each lacked an efficient self-cleansing masticatory apparatus.

DISCUSSION

This census of salivary microorganisms has provided a broad coverage of a number of categories, and yet it is limited in scope. Other categories can be named which might have been included, although it is probable that for most of these a suitable selective or differential medium awaits description. The Corynebacterium and total Micrococcus categories exemplify 2 such groups. Previous to and during a considerable part of this study, various modifications of tellurite-containing media were used in attempts to establish the census of Corynebacterium. On these trial media, micrococci and Neisseria occurred at least as commonly as Corynebacterium and could not be distinguished. The present study was near completion when Rogosa and Shiota[20] reported a selective medium for Corynebacterium, and this medium was not included. For a census of total micrococci a reliable selective or differential medium is needed; only an estimate of this population was possible in this report.

Direct microscopic counts as well as cultural counts have been reported for saliva as well as plaque and carious material. Cultural quantitative estimations from saliva or other oral material undoubtedly fail to provide an accurate census, principally because favorable growth conditions for all organisms

TABLE II

BACTERIAL CATEGORIES WHICH WERE SIGNIFICANTLY DIFFERENT FOR THE INDIVIDUAL SUBJECT AND THE DENTAL STATUS OF EACH SUBJECT

SUBJECT	CATEGORIES IN WHICH THE MEAN FOR THE SUBJECT DIFFERED SIGNIFICANTLY FROM CATEGORY MEAN FOR THE GROUP		DENTAL STATUS
	LOW COUNT	HIGH COUNT	
I	Total aerobes Neisseria Starch hydrolyzers	None	High past caries activity; healthy gingivae, no calculus
II	Leptotrichia H_2S producers	None	Moderate past caries activity, presently inactive; healthy gingivae, no calculus
III	Leptotrichia	None	Slight past caries activity, presently inactive; healthy gingivae, no calculus
IV	Leptotrichia	None	High past caries activity, presently slightly active; healthy gingivae, no calculus
V	Leptotrichia	None	High past caries activity; four posterior teeth missing; defective fixed replacement for missing anterior tooth; slight gingivitis and calculus
VI	None	Lactobacillus	Active carious lesions present and high past caries activity; slight gingivitis and calculus
VII	None	None	No examination made
VIII	None	H_2S producers Coliforms	Moderate plaque accumulations and past caries activity, presently caries active; moderate gingivitis and calculus deposits
IX	None	Micrococci Neisseria Fusobacterium H_2S producers	Caries immune; advanced periodontitis and moderately heavy calculus deposits
X	None	Total aerobes Total anaerobes Total streptococci *S. salivarius* Starch hydrolyzers Veillonella Leptotrichia	Moderate past caries experience and presently caries active; slight gingivitis and calculus deposits
XI	None	Total aerobes Total anaerobes Total streptococci *S. salivarius* Veillonella Neisseria Starch hydrolyzers	High past caries experience and presently caries active; slight gingivitis and moderate calculus deposits
XII	Leptotrichia	None	High past caries experience but presently caries inactive; healthy gingivae and no calculus
XIII	None	Neisseria	Slight past caries experience; slight gingivitis, no calculus
XIV	Leptotrichia Starch hydrolyzers	None	Caries immune; early periodontosis, no calculus, marked occlusal abrasion

are not established. Using direct microscopic counts, dead as well as viable bacteria are counted, and total counts by this method are higher than counts obtained by culture. An even greater discrepancy between counts by the 2 methods is introduced by the fact that direct counting will include each organism of a chain or cluster, whereas in cultural counts the total chain or cluster probably will be manifest as only one colony and therefore will be counted as one organism. Both counting methods have much to recommend them, and each will find usefulness. Counts by either method should be evaluated in proper perspective, and a comparison of counts would seem only rarely justified. The true population probably lies somewhere between values obtained by the 2 methods.

The number of subjects contributing saliva for this investigation was not large. Preliminary calculations based on data from 11 of the 14 subjects showed only minor deviations when compared to complete data. This would indicate that a further analysis of a larger number of subjects and specimens would probably not yield data commensurate with the effort. Furthermore, inherent errors in the methods, e.g., low Fusobacterium counts, would not be corrected by the collection of additional data using the same procedure.

There were certain advantages in working with a group of this size. For example, it was possible for an investigator to form a mental picture of each mouth while reviewing the data. In addition, a close personal relationship of the investigators with the subjects enabled consideration of the data in terms not frequently analyzed.

One of the aims of this investigation was to determine, if possible, which categories were most stable from specimen to specimen for all subjects. Three categories appeared to be more stable than the others; these were the Veillonella, total anaerobes, and total aerobes. The use of these groups might be preferred in studies employing bacterial counts to evaluate the effect of experimental tools (mouthwashes, dentifrices, etc.) on the oral population.

The present study is believed to be the first in which a census of Leptotrichia in saliva has been established by cultural methods. Since the tentative identification was based on colonial and cell morphology, further examination of this group is planned.

This investigation was not designed to study the relationship of a particular dental disease as reflected by the salivary flora. Accordingly, the subjects represented a random sample of university personnel. The experiment would have had value, although considerably less, had the mouths not been inspected. However, considering the census data along with the dental status, 5 categories of organisms were observed to constitute an index of gingival health and tendency toward formation of calculus.

SUMMARY

A census of 14 bacterial groups has been obtained by cultural methods from unstimulated saliva of 14 adults. The saliva of each subject was examined on 10 separate occasions.

The group mean for the various categories may be expressed as high, intermediate, and low populations. High population means ($n \times 10^6$ per ml. saliva) were: total anaerobes 110, total aerobes 40, total streptococci 18, Veillonella 17, *S. salivarius* 11, starch hydrolyzers 5, hydrogen sulfide-producers 2, and Neisseria 2. A total micrococcus population was estimated to be about that of the Neisseria. Intermediate population means ($n \times 10^3$ per ml. saliva) were: fusobacteria 56, lactobacilli 35, salt-tolerant micrococci 5, and Leptotrichia 3. Low populations ($n \times 10^2$ per ml. saliva) were: Candida 2 and coliforms 1.

The Veillonella, total anaerobes and total aerobes were categories in which the least fluctuation occurred from examination to examination for all of the subjects.

No statistical differences were found between vegetarians and non-vegetarians for any of the categories.

Certain characteristics were identified with subjects exhibiting low counts as opposed to those exhibiting high counts. Thus, females had lower counts than males, slow spitters had lower counts than fast spitters, and nonsmokers or occasional smokers had lower counts than smokers.

Population counts of salt-tolerant micrococci, Neisseria, Leptotrichia, fusobacteria, and hydrogen sulfide-producers provided an index of gingival health. Subjects with statistically significant high counts in one or more of these categories had varying degrees of gingivitis and calculus deposits, whereas subjects with significantly low counts had clinically healthy gingiva and little or no calculus.

The authors wish to express their grateful appreciation to Dr. Sarang Iyer for his cooperation in contacting the vegetarians and to Dr. Lloyd A. Knowler, Professor of Mathematics and Astronomy, State University of Iowa, for his suggestions concerning the statistical treatment of the data.

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ELASTIC AND MECHANICAL PROPERTIES OF HUMAN DENTIN

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THE elastic and mechanical properties of dentin were studied as early as 1895 by Black[1] who measured the compressive strengths of cubes of dentin and reported that a cube, 0.08 inch square, had an average compressive strength of 37,200 psi and an elastic deformation of 2.09 per cent when subjected to a load of 100 pounds (elastic modulus ~ 0.8×10^6 psi). Peyton, Mahler, and Hershenov[2] reported average values of 23,400 psi, 36,100 psi, and 1.67 $\times 10^6$ psi for the proportional limit, compressive strength, and elastic modulus, respectively. Stanford, Paffenbarger, and Kumpula[3] reported average values for these properties of 25,100 psi, 50,400 psi, and 4.1 $\times 10^6$ psi, respectively. Neumann and Di Salvo[4] reported values from 1.1 to 1.7 $\times 10^6$ psi for the elastic modulus using large transverse sections of teeth. The values for the proportional limit and compressive strengths of dentin were in reasonable agreement, but those for the elastic modulus differed widely.

The difficulties involved in handling small specimens of dentin and the problems connected with correcting the deformation used in the calculation of the elastic modulus values possibly account for the differences obtained in the various studies. It was felt that a critical re-evaluation of the technics and procedures used to obtain the elastic modulus, with particular emphasis on flow properties, would help to establish a better experimental value for the elastic modulus as well as the other physical properties of dentin.

EXPERIMENTAL

Equipment.—The equipment used to measure the elastic properties of dentin was the same as that described by Peyton, Mahler, and Hershenov,[2] except for the compression testing machine and the type of steel plungers used. The stress was applied to the samples by a Riehle testing machine. The load scales of the testing machine were calibrated for loads in compression with a recently calibrated dynamometer ring. The calibration data for the 250 lb. scale, which was used for most of the measurements, showed that the load indicator was accurate to ± 0.5 per cent at the low end of the scale and to ± 0.1 per cent for the high portion of the scale. The actual error was generally ± 0.2 lb. over the entire scale. The accuracy on this and higher scales was greater than reading errors, and, therefore, the recorded scale readings were used directly in the calculation of the stress.

This report represents the partial results of studies supported by Contract No. D-462 between the U. S. Public Health Service, National Institutes of Health, and the University of Michigan.

Received for publication Dec. 20, 1957.

The steel plungers were prepared from cylindrical rods with half of the rod shaped to form a bar, 0.175 inch square. The square end was ground smooth, and 2 of these plungers, supported by a steel frame, were placed end to end with the specimen between them to form the compression unit. Prior to making any compression measurements the two steel ends were placed together and a stress applied. The stress was increased to a point just below the proportional limit of the steel plungers (2,500 lb.) and then relieved. This operation was repeated several times, and for all subsequent measurements the plungers were always used in the same relative position.

Specimen Preparation.—Blanks, in the shape of triangular prisms, were prepared from human first and second molars by sectioning and grinding. These blanks were then placed in a machine lathe and turned down into the shape of a cylinder. The ends of the specimens were finished flat and parallel by placing the cylinders in a hole in the center of a split brass disk, ¾ inch in diameter, and polishing with 240A, 400A, and 600A Norton Tufbak Speed wet paper supported on a plate glass slab. Compression specimens 0.100 inch in diameter and 0.100 to 0.400 inch long were prepared in this manner.

Compression specimens of steel (SAE 1020), aluminum (24 S-T), and polystyrene (Jectron) were also prepared on the machine lathe, their dimensions being 0.170 inch in diameter and 0.250 to 0.300 inch long. In addition, standard ¼ inch diameter tensile specimens were prepared from the 3 materials listed above.

Procedures.—The elastic moduli in compression of the various materials were determined from the slopes of the stress-strain curves within the elastic region. The necessary stress-strain curves were obtained by taking simultaneous readings of the stress, recorded on the testing machine, and of the strain, indicated on the Tuckerman Optical strain gauges. The loading was not interrupted during the experiment, and the stress and strain measurements were taken during loading. The rate of load application was usually 3,400 psi per minute, but several experiments were conducted at 34,000 psi per minute (see Table II).

Since most of the dentin specimens were approximately 0.20 inch long and the strain gauges, as normally used, were 1 inch in length, the deformation recorded included the deformation for about 0.80 by 0.175 by 0.175 inch of steel. Of course, corrections were made in the calculations for this contribution. The Tuckerman Optical strain gauges are designed so that they can also be used as ¼ inch gauges.[5] In several instances, dentin specimens longer than 0.25 inch were obtained, and in these cases the ¼ inch gauge length was used which spanned a continuous cylinder of dentin. In these cases no correction for the deformation of steel was required.

The elastic moduli in tension for steel, aluminum, and polystyrene were determined on the standard tensile specimens using a 1 inch gauge. The strain gauge extended across a continuous section of material, again making corrections to the deformation values unnecessary.

RESULTS AND DISCUSSION

In order to establish the reliability of the modulus of elasticity obtained in compression with small samples of dentin, small cylinders of steel, aluminum, and polystyrene were prepared and the elastic moduli were determined. The accuracy of these measurements was established by comparing them with experimentally determined elastic moduli values in tension and with accepted published values for these materials.

Table I contains average experimental values for the elastic moduli of steel, aluminum, and polystyrene determined both in compression and in tension. These values are in good agreement with each other and are also in accord with published results. Thus, the elastic modulus values of dentin determined in compression should represent experimentally satisfactory results. This is not to imply that the elastic modulus of dentin in compression will equal the elastic modulus in tension since this is only true for isotropic solids, and differences in these values can exist in the case of laminated structures.

TABLE I
MODULUS OF ELASTICITY VALUES DETERMINED IN COMPRESSION AND TENSION

MATERIAL	EXPTL. ELASTIC MODULUS, COMPRESSION $\times 10^6$ PSI	EXPTL. ELASTIC MODULUS, TENSION $\times 10^6$ PSI	ACCEPTED ELASTIC MODULUS,* TENSION $\times 10^6$ PSI
Steel (SAE 1020)	29.7	29.4	29.0
Aluminum (24 S-T)	10.2	10.5	10.3
Polystyrene (Jectron)	0.53	0.54	---

*Values taken from Hoyt, S. L., *Metals and Alloys Data Book*, New York, 1943, Reinhold Publishing Corporation.

The values recorded in the first column of Table II represent the various experimental elastic moduli for dentin specimens determined with a 1 inch strain gauge and corrected for the effect of the elastic modulus of steel. The values ranged from 1.84 to 2.41 $\times 10^6$ psi, resulting in an average value of $2.10 \pm 0.18 \times 10^6$ psi. The reproducibility of a single measurement was checked by making two separate determinations of the elastic modulus on a single sample. In each case a value of 2.02×10^6 psi was obtained.

Since the ends of the dentin specimens were smaller than the square ends of the compression plungers, the surface of the plungers will deform to some extent when stress is applied, and this will result in a false strain reading. A correction for this effect has been proposed by Stanford, Paffenbarger, and Kumpula,[3] and the second column in Table II lists the elastic modulus values with this type of correction applied. The correction amounted to approximately 0.18×10^6 psi for the size specimens and plungers used in this research.

The direction of the dentin specimen in relationship to the tooth, the rate of loading on the dentin specimens (from 3,400 to 34,000 psi/min.), and the dryness of the dentin did not have any measurable effect on the elastic modulus. The length/diameter ratio of the dentin specimens, however, did appear to have some effect on the elastic modulus but, in general, it was independent of the length/diameter ratio in the range from 1/1 to 2.5/1. For ratios between 1/1 and 0.5/1, the elastic modulus values were lower and appeared to be

a direct function of the length. Two reasons for this observation are: (1) an actual decrease in the elastic modulus as the specimen shape changes from a cylinder to a plate, and (2) a lower slope of the stress-strain plot (lower modulus) caused by non-parallel ends of the specimen. This second factor would become more important the shorter the specimen, since the error introduced into the strain measurement would then be a larger percentage of the total strain. Thus, in the low length/diameter range of 0.5/1 to 1/1 it is felt that the second factor is the major reason for the low elastic modulus values.

TABLE II
ELASTIC MODULUS OF DENTIN

1 INCH STRAIN GAUGE*			¼ INCH STRAIN GAUGE†	
ELASTIC MODULUS, E_y, CORRECTED FOR E_s OF STEEL PLUNGERS $\times 10^6$	ELASTIC MODULUS CORRECTED FOR E_s AND FOUNDATION EFFECTS $\times 10^6$	ELASTIC MODULUS CORRECTED FOR E_s AND F.E. AND CALC. FROM DECOMPRESSION CURVE $\times 10^6$	ELASTIC MODULUS $\times 10^6$	ELASTIC MODULUS CORRECTED FOR RETARDED ELASTIC DEFORMATION $\times 10^6$
1.93‡	2.13		2.20‖	2.57
2.41§	2.58		2.20‖	2.57
2.02‖	2.20		2.52	2.85
2.02‖	2.20		2.41	2.70
1.97	2.15			
2.39	2.57			
1.90‡, §	2.08	2.41		
1.84	2.02	2.35		
2.38¶	2.56			
2.10 ± 0.18 Avg.	2.28 ± 0.18 Avg.		2.33 Avg.	2.67 Avg.

*The specimens were 0.20-0.25 inch long and therefore 0.75-0.80 inch of the length of the strain gauge was steel.

†The specimens were 0.35-0.40 inch long and therefore the entire length of the 0.25 inch strain gauge was a continuous specimen of dentin.

‡The long axis of the specimen was perpendicular to the occlusal surface; in the remaining specimens the long axis was parallel to the occlusal surface.

§The rate of loading was 34,000 psi/min.; the rate of loading for the remaining specimens was 3,400 psi/min.

‖These values represent separate determinations of the elastic modulus on identical specimens.

¶Specimen was dried for 30 hours at 105° C. prior to determining E_y.

The elastic modulus values reported in the fourth column of Table II were obtained on a continuous piece of dentin using a ¼ inch strain gauge. The need of correcting for the elastic modulus of the steel plungers and foundation effects was, therefore, avoided. The values varied from 2.20 to 2.52 $\times 10^6$ psi, resulting in an average value of 2.33 $\times 10^6$ psi which is in good agreement with 2.28 $\times 10^6$ psi, the average obtained using specimens less than ¼ inch long and a 1 inch strain gauge. The remaining 2 columns of Table II (3 and 5) represent elastic modulus values corrected for the retarded elastic deformation or recoverable flow of dentin. This correction increases the elastic modulus values approximately 0.34 $\times 10^6$ psi. Since this phenomenon will be treated in greater detail later in this section, the discussion of these results will be deferred until that time.

Peyton, Mahler, and Hershenov[2] previously published a value of 1.67 $\times 10^6$ psi for dentin and Stanford, Paffenbarger, and Kumpula[3] reported a value of 4.1 $\times 10^6$ psi. No corrections were made on the former value and, thus, this value would be increased to approximately 2.3 $\times 10^6$ by applying the average corrections for the elastic modulus of steel, foundation effects, and recoverable

flow. This corrected value is then in fair agreement with the average value of approximately 2.7 $\times 10^6$ psi, which will be reported later in detail. The disagreement with the value reported by Stanford, Paffenbarger, and Kumpula may be due to their empirical method for correcting the experimental compression modulus values.

The proportional limit, defined as the stress at the point where the stress-strain curve ceases to be linear, and the ultimate compressive strength of a number of dentin specimens are tabulated in Table III. The proportional limit for dentin varied from 20,200 to 30,100 psi, the average value being 24,200 ± 2,900 psi. The ultimate compressive strength values ranged from 38,100 to 49,400 psi, yielding an average value of 43,100 ± 3,600 psi; these results did not show any correlation with the corresponding proportional limits. Black,[1] Peyton, Mahler, and Hershenov,[2] and Stanford, Paffenbarger, and Kumpula[3] have reported average values of 37,200, 36,100, and 50,400 psi, respectively, for the compressive strength of dentin and the latter two groups have published average values of 23,400 and 25,100 psi, respectively, for the proportional limit of dentin. Considering the wide range of compressive strengths obtained in any one investigation, the average values are in reasonable agreement. No absolute basis for a choice between these values is possible; however, it is more difficult to discount high than low results since errors in compressive strength measurements are most likely to be caused by faulty specimen preparation and these errors would yield low rather than high values. The rate of load application used in the various researches would also have an effect on the compressive strength of dentin. The higher averages of 50,400 and 43,100 psi are probably closer, therefore, to the true figure for dentin than values of 36,100 and 37,200 psi. The average proportional limit of 24,200 psi obtained in this research compares well with, and is about midway between, the literature values of 23,400 and 25,100 psi.

TABLE III
PROPORTIONAL LIMIT AND ULTIMATE COMPRESSIVE STRENGTH OF DENTIN

PROPORTIONAL LIMIT (PSI)	ULTIMATE COMPRESSIVE STRENGTH (PSI)
23,000	38,100
20,200	40,000
24,600	41,800
30,100	44,600
24,400	43,800
22,800	49,400
26,000	41,800
20,200	43,300
26,300	45,000
26,400*	52,000*
24,200 Average	43,100 Average

*Specimen was dried for 30 hours at 105° C. prior to making measurements and values were not included in the average.

In the process of determining the stress-strain plots for dentin, it was observed that, if the stress was maintained at a constant value, the strain would continue to increase. It was of interest to study this effect further and to establish whether this flow represented permanent deformation and what effect the flow would have on the calculated elastic modulus.

Fig. 1 shows the effect of loads below and above the proportional limit on the flow properties of dentin. In the first case, the load of 110 lb. was applied to the specimen as rapidly as possible, and the autocollimator reading was determined at various times (the autocollimator readings were not converted to strain since only relative values were needed to study the flow properties; to convert to strain the autocollimator reading should be multiplied by 0.0013 inch/inch). As shown in the graph, the strain increased with time when the load was maintained at 110 lb. After 20 minutes the load was relieved and the major portion of the deformation, the Hookian elastic deformation (ϵ_h), was recovered immediately. The remaining deformation, the retarded elastic

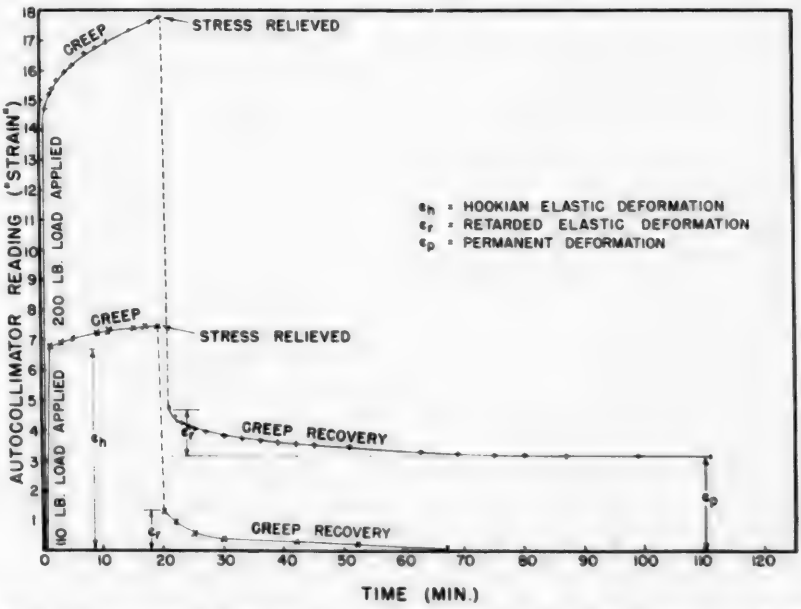

Fig. 1.—Deformation of dentin under stress above and below the proportional limit.

deformation (ϵ_r), however, was not completely recovered until 50 minutes later. The same load cycle was used with a 200 lb. load applied on the dentin specimen and a similar shaped curve was obtained (the stress in this case was above the proportional limit). A correspondingly larger value for ϵ_h was obtained and, although the value for ϵ_r was somewhat larger than for the lower load, the relation of the values indicates that the retarded elastic deformation was load, as well as time, dependent. After the load was relieved, however, a portion of the deformation was not recovered, and ϵ_p represents this permanent deformation.

In order to establish clearly the fact that the flow observed in dentin was true flow and not caused by instrumental difficulties, a compression specimen of aluminum was subjected to compression and decompression. A single stress-strain curve was obtained from the compression and decompression values, thus establishing the validity of the results on dentin.

If the elastic modulus is considered to be represented by the ratio of the stress to the Hookian elastic deformation, then some means of correcting for the retarded elastic deformation should be used. Two methods for practically eliminating the contribution of the retarded elastic deformation were to calculate the elastic modulus from: (1) the stress-strain curve obtained on relieving the compressive stress on a dentin specimen, or from (2) the stress-strain curve obtained on the second or higher compression cycle. These procedures are illustrated in Fig. 2. The stress-strain curves were obtained on a dentin specimen less than 0.25 inch long, for which reason 1 inch strain

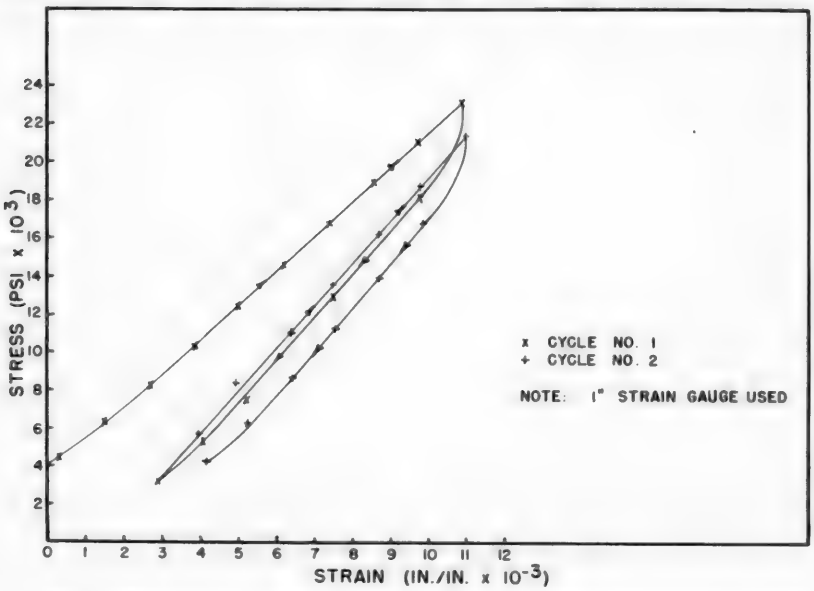

Fig. 2.—Stress-strain curves of dentin for two compression-decompression cycles.

gauges were used. The stress was increased from an initial stress of 4,000 psi to about 23,000 psi, which was below the proportional limit for this specimen; the stress was then decreased to approximately 3,000 psi, increased to 22,000 psi, and decreased again to about 4,000 psi. During these cycles, simultaneous readings of load and deformation were taken, permitting the calculation of the stress-strain curves in compression and decompression.

It can be observed that the compression curve for cycle 1 was not linear below a stress of 7,000 psi (Fig. 2). Also the slope of the linear portion of the compression curves for cycle 1 was less than the slope of the compression curve for cycle 2 or the decompression curves for cycle 1 or 2. The decompression curves for cycle 1 or 2, likewise, did not coincide with the compression curves for cycle 1 or 2, respectively. The compression curve for cycle 2 did not coincide with the decompression curve of cycle 1, with the former having higher stress values for corresponding strain values. The slopes of the compression curve for cycle 2 and the decompression curves for both cycles 1 and 2 were equal.

These five observations from Fig. 2 may be explained in the following manner: (1) The non-linearity of the first stress-strain curve was due to not having the ends of the dentin specimen exactly parallel. (2) The slope was lower for the first compression cycle because of the contribution to the strain of the retarded elastic deformation, and to some extent the non-parallel ends of the specimen. (3) The decompression curves did not coincide with the compression curves because the retarded elastic deformation did not have sufficient time to recover in the 6 to 8 minutes required to traverse the decompression range, as revealed by the curve. (4) The compression curve for cycle 2 did not coincide with the decompression curve of cycle 1 because some of the retarded elastic deformation was being recovered even though the stress was increasing. (5) The slopes of the compression curves for cycle 2 and the decompression curves for cycles 1 and 2 were equal because the effects of non-parallel ends and retarded elastic deformation had been largely avoided as influencing factors.

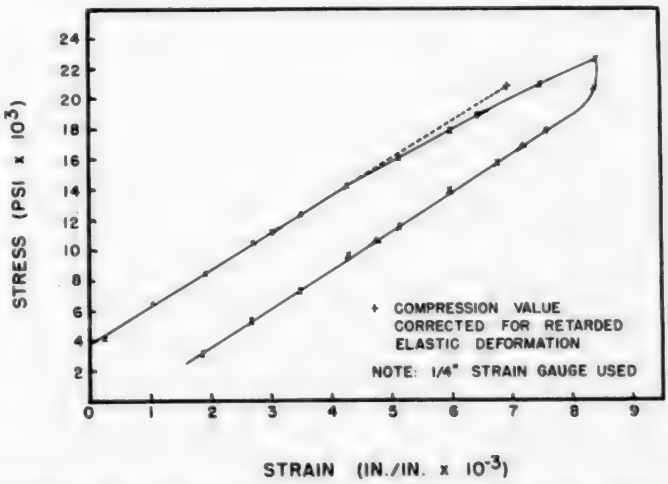

Fig. 3.—Stress-strain curves in compression and decompression for a continuous dentin specimen.

When a dentin specimen longer than 0.25 inch was used with a ¼ inch strain gauge, the effect of the specimen ends not being parallel was eliminated. The stress-strain curve in compression and decompression for this type of system is shown in Fig. 3. It is apparent that the stress-strain curve in compression at low stress values was linear and deviated from linearity at about 14,000 psi. This deviation was caused by the retarded elastic deformation. The decompression curve was linear throughout the main portion of its length and was parallel to the initial portion of the stress-strain curve in compression, again because the retarded elastic deformation did not have sufficient time to recover. It should be noted that the strain at 20,500 psi has been corrected for this retarded elastic deformation. This correction was determined by relieving the load as rapidly as possible to the original initial load and observing

the value of the retarded elastic deformation included in the total deformation at 20,500 psi stress. When the early stress-strain points and the corrected value of the strain at 20,500 psi stress were used to draw the stress-strain curve in compression, a straight line was obtained which was parallel to the decompression curve. The same elastic modulus value was, therefore, obtained by either correcting the compression curve or by directly calculating the slope of the decompression curve. It should be noted that the rate of loading is unimportant if the correction for retarded elastic deformation is made.

The best values for the elastic modulus of dentin are, therefore, those listed in columns 3 and 5 of Table II. Unfortunately, stress-strain curves in decompression were determined for only 2 specimens using the 1 inch strain gauge (column 3), and they yielded an average elastic modulus of 2.38 $\times 10^6$ psi. If it is assumed that the values in column 3 are on the average 0.33 $\times 10^6$ psi larger than those in column 2 (as indicated by data), the average value for column 3 would be approximately 2.6 $\times 10^6$ psi. Elastic modulus values determined on dentin specimens using ¼ inch strain gauges and corrected for the retarded elastic deformation ranged from 2.57 to 2.85 $\times 10^6$ psi (column 5) and yielded an average value of 2.67 $\times 10^6$ psi. Thus, this research indicates that a good average value for the elastic modulus of dentin is 2.65 $\times 10^6$ psi.

SUMMARY AND CONCLUSIONS

The proportional limit and the ultimate compressive strength of dentin have been found to have average values of 24,200 and 43,100 psi, respectively.

The corrected elastic modulus of dentin was independent of the direction from which the specimens were taken from the teeth and from the rate of loading the specimen. The elastic modulus was also found to be independent of the length/diameter ratio for the specimen within the limits of 1/1 to 2.5/1.

Comparable values for the elastic modulus of dentin were obtained regardless of whether or not the deformation used in the calculation was determined with a strain gauge spanning a continuous dentin specimen or a combination of dentin and steel, when proper corrections were made.

The total deformation in a dentin specimen below the proportional limit consisted of pure and retarded elastic deformation. Since this latter deformation was a function of time as well as load, it must be substracted from the total deformation before a correct elastic modulus can be calculated.

The average corrected elastic modulus of dentin was found to be between 2.4 and 2.7 $\times 10^6$ psi.

The assistance of B. F. Caswell and R. G. Smith in the experimental work is gratefully acknowledged.

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INTRAPERITONEAL INJECTIONS OF CARBOHYDRATES TO SUCKLING RATS, AND SUBSEQUENT DENTAL CARIES

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PREVIOUS work has shown that when a carbohydrate is given to caries susceptible rats during the suckling period in addition to mother's milk, a subsequent increase in caries occurs.[1, 2] Since the molars had not errupted in the mouth until the end of the suckling period,[3] this effect must have been other than local. This investigation was carried out to determine whether this increase in caries was the result of a digestive disturbance or mainly systemic in nature..

PROCEDURE

Nineteen animals from 4 litters were divided into 2 groups of littermates on the day following birth. Ten animals received a 20 per cent glucose solution injection 3 times a day from the day following birth through the fourth day. The amount of carbohydrate administered to both groups was approximately 1 mg. per gram of body weight. All animals were nursed by their mothers ad libitum through the twenty-first day, at which time they were weaned and placed on a synthetic cariogenic diet of the following composition: casein 24, sucrose 65, salts U.S.P. XIV 4, corn oil 5, dehydrated whole liver 4, vitamin mix 2,[4] choline 0.2 parts. In addition to this diet, all animals received vitamin A and D drops once a week. At the end of 13 weeks the animals were sacrificed and the teeth were examined for caries. The molars were examined by grinding successive planes parallel to the occlusal surface. Lesions were scored by the method of Shaw[5] (Table I).

TABLE I

CARIES FOLLOWING THE ADMINISTRATION OF 20% GLUCOSE, ORALLY AND BY INJECTION

METHOD OF ADMINISTRATION	NUMBER OF ANIMALS	CARIES SCORE	SIGNIFICANCE
Oral	9	9.1	
Injection	10	16.2	0.02

The injection of a mixture of equal parts of glucose and fructose had a similiar effect in causing a subsequent increase in caries. Four litters, totaling 34 animals, were divided into 3 groups of littermates at 3 days of age. Eleven animals received a 20 per cent mixture of glucose and fructose solution by intraperitoneal injection 3 times a day from the third day through the twenty-first

This study was supported by a grant from the National Institutes of Health DA254C, United States Public Health Service.

Received for publication Dec. 21, 1957; revised by authors March 27, 1958.

day, in a dosage of 1 mg. per gram of body weight. Twelve animals received the 20 per cent mixture of glucose and fructose orally, 3 times a day, in approximately the same dosage from the third day through the twenty-first day. The third group of 11 animals when weaned were placed on the cariogenic diet previously mentioned. After 13 weeks the animals were sacrificed and their teeth examined (Table II).

TABLE II

CARIES FOLLOWING THE ADMINISTRATION OF A 20 PER CENT MIXTURE OF GLUCOSE AND FRUCTOSE, ORALLY AND BY INJECTION

METHOD OF ADMINISTRATION	NUMBER OF ANIMALS	CARIES SCORE	SIGNIFICANCE
Injection	11	23.2	p 0.03
Oral	12	22.2	p 0.02
Control	11	15.1	

The following investigation was conducted to determine what effect the addition of vitamins B_6 and C to the injected solution of carbohydrate might have on subsequent dental caries. Forty-seven animals were divided into 3 groups of littermates, 3 days following birth. Seventeen animals were injected with a 20 per cent solution of equal parts by weight of glucose and fructose 3 times a day from the third day through the twenty-first day. The dosage given was 1 mg. of carbohydrate per gram of body weight. The second group of 15 animals received injections of a 20 per cent solution of equal parts by weight of glucose and fructose, 1 mg. per gram of body weight, plus 0.1 mg. of vitamin C per 10 grams of body weight, 3 times a day from the third day through the twenty-first day. The third group of 15 animals received injections of the 20 per cent mixture of equal parts by weight of glucose and fructose, in a dosage of 1 mg. per gram of body weight, plus 0.1 mg. of vitamin B_6 per 10 grams of body weight, 3 times a day from the third day through the twenty-first day of age.

All animals were weaned at 21 days of age and placed on the synthetic cariogenic diet previously outlined. After 13 weeks all animals were sacrificed and their molars examined (Table III).

TABLE III

DENTAL CARIES FOLLOWING THE ADMINISTRATION OF 20 PER CENT MIXTURE OF GLUCOSE AND FRUCTOSE PLUS THE ADDITION OF VITAMIN C AND B_6

SOLUTION INJECTED	NUMBER OF ANIMALS	NUMBER OF CARIES FREE ANIMALS	CARIES SCORE	SIGNIFICANCE
20% glucose, fructose	17	1	16.9	
20% glucose, fructose plus vit. C	15	5	8.0	p 0.05
20% glucose, fructose plus vit. B_6	15	5	6.5	p 0.02

It would appear that the injection of a monosaccharide intraperitoneally during the calcification of the molars caused a subsequent increase in caries over that in littermates not so treated. This increase in subsequent caries by injection is comparable to the increase in subsequent caries following the oral administration of a carbohydrate over that in littermates not so treated.[1, 2]

The administration of a carbohydrate by mouth is subject to variations which are not encountered by intraperitoneal injections. The amount of carbohydrate administered is of importance. In a study in which an artifical formula was fed to the young during the last third of the suckling period, an increase in the amount of a given carbohydrate caused a corresponding increase in caries.[6] The milk of rats contains about 3 per cent of carbohydrate.[7]

The reduction in subsequent caries in those animals receiving the vitamins C and B_6 in addition to the 20 per cent of glucose and fructose solution might indicate that the administration of the added carbohydrate alone was diluting the diet received by the young. Vitamin C has been shown to be necessary during the calcification of the teeth.[8] Although rats are able to synthesize vitamin C, at this age this may not be possible in adequate amounts under adverse conditions. The milk of rats contains some vitamin C normally.[7] A relationship between vitamin B_6 deficiency and dental caries has been proposed.[9] Vitamin B_6 is also found in rats' milk.[8]

SUMMARY

The injection of a monosaccharide to suckling rats 3 times a day during the suckling period produced a subsequent increase in caries. This increase in caries by injection of various monosaccharides is similar to the increase produced by the oral administration of the same carbohydrate.

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THE EFFECT OF PAROTID DUCT EXCISION AND PAROTID GLAND EXTIRPATION ON DENTAL CARIES INCIDENCE IN HUNT-HOPPERT CARIES-RESISTANT RATS

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AN INCREASED production of carious lesions has been reported in the laboratory rat, the cotton rat, and the Syrian hamster as a result of the extirpation of some or all of the principal salivary glands under varying experimental conditions.[1-8] Cheyne[2] found that the removal of either the parotid glands or the submaxillary and major sublingual, or the parotid and submaxillary glands caused extensive increases in the initiation and production of dental caries in rats maintained on the Hoppert-Webber-Canniff coarse-particle, caries-producing diet.[9] Schwartz and Shaw,[10] in studies to clarify the role of the individual salivary glands, removed single pairs or combinations of pairs of salivary glands from rats that were being maintained on a refined, high-sucrose cariogenic ration. The removal of either the parotid glands or the submaxillary glands resulted in highly significant increases in dental caries incidence as compared to the control intact animals. The removal of the major sublingual or the extraorbital lacrimal glands did not produce significant increases in caries incidence.

However, when Keller, Hunt, and Hoppert[11] removed the parotid ducts and parts of the parotid glands from inbred Hunt-Hoppert caries-susceptible and caries-resistant albino rats maintained on the Hoppert-Webber-Canniff ration, they did not observe any appreciable dental caries increase. In view of this unexpected difference from earlier observations, we obtained adult rats from the Hunt-Hoppert caries-susceptible and caries-resistant strains to develop small colonies for the production of offspring to be used in our laboratory to compare the effect of their procedure of excision of the parotid ducts versus the influence of our procedure for extirpation of the parotid glands.

EXPERIMENTAL

The rats used in these experiments were bred in our laboratory from representatives of the caries-resistant Hunt-Hoppert strain that were being maintained throughout their reproductive lives on Harvard cariogenic diet No. 700.[12]

These studies have been supported in part by Research Grant D-373 of the National Institute of Dental Research, United States Public Health Service.

Received for publication Dec. 24, 1957.

Thus the offspring from this strain at the initiation of these experiments had a dietary background that was comparable to that of the Harvard caries-resistant and caries-susceptible strains used in previous studies in our laboratories. Hunt-Hoppert caries-susceptible animals were not included in the present studies because of the severe breeding problems that have been associated with this strain in our laboratories, irrespective of whether they were maintained on the Hoppert-Webber-Canniff diet or Harvard diet No. 700.

Two separate experiments were carried out. In the first, the rats were weaned at 21 days of age and placed in individual wire-bottom cages and provided with ration No. 700 and tap water ad libitum. The rats were divided with careful attention to equitable littermate distribution into 3 groups consisting of 17, 16, and 15 subjects. The first group was composed of the controls which were not operated upon throughout the entire experiment. The parotid ducts of the rats in the second group were tied and then severed, while the parotid glands of the rats in the third group were extirpated surgically. All the operations were carried out under ether anesthesia. All operative technics were done on the same day when the rats were between the thirtieth to the thirty-fifth day of age. The average weight of the rats was approximately 70 grams. The second study was conducted in the same way with the same experimental design, with the exception that the Hoppert-Webber-Canniff ration was substituted for ration No. 700 throughout. There were 17 rats in each group.

At the end of 115 days after operation, the rats were killed and an autopsy of the salivary glands performed. Selection of this time interval was based on clinical determinations of the first appearance of grossly visible lesions in the control rats. The parotid, submaxillary, and major sublingual glands were weighed in order to calculate the ratios of gland weight to body weight of the operated rats as compared to the control animals.

The head of each rat was then placed in 95 per cent ethanol for about 48 hours. After removal of the soft tissues from the jaws, the teeth were evaluated for caries activity by the scoring system devised for the cotton rat.[13]

RESULTS

In the first experiment, excision of the parotid ducts of the caries-resistant Hunt-Hoppert rats fed ration No. 700 resulted in a highly significant increase in caries incidence compared to control animals in respect to the average number of carious molars, average number of carious lesions, and the average extent of carious lesions (Table I). In almost every rat in this group, the parotid glands had atrophied to the point where no identifiable remnants were visible. Surgical extirpation of the parotid glands also produced a highly significant increase in caries. No significant difference was observed between the amount of caries in the group of rats where the parotid ducts were tied and severed and the group of rats where the parotid glands were entirely removed. From these data, it is clear that the influences of the 2 operative procedures had the same end result.

TABLE I

THE INFLUENCE OF PAROTID DUCT EXCISION OR PAROTID GLAND EXTIRPATION ON DENTAL CARIES INCIDENCE OF RATS FED RATION NO. 700 OR THE HOPPERT-WEBBER-CANNIFF DIET

GROUP	NO. OF RATS	JAW SCORED	NO. OF CARIOUS MOLARS			NO. OF CARIOUS LESIONS			EXTENT OF CARIOUS LESIONS		
			AVG.	SEM*	CR†	AVG.	SEM*	CR†	AVG.	SEM*	CR†
Experiment 1—Ration No. 700											
Control	17	Maxilla	0.6	0.3		0.7	0.4		1.6+	0.9+	
		Mandible	2.5	0.4		3.5	0.9		8.4+	2.7+	
		Both	3.1	0.7	6.7	4.2	1.2	7.3	10.0+	3.6+	6.4
Parotid duct tied and severed	16	Maxilla	3.4	0.4		5.4	0.7		15.9+	2.1+	
		Mandible	5.5	0.2		11.9	0.8		33.8+	3.0+	
		Both	8.9	0.5	0.6	17.3	1.4	0.6	49.7+	5.1+	0.5
Parotid gland extirpation	15	Maxilla	3.9	0.2		5.5	0.4		15.5+	1.7+	
		Mandible	5.4	0.2		10.8	0.6		30.8+	2.5+	
		Both	9.3	0.4	7.8	16.3	1.0	8.1	46.3+	4.0+	6.7
Experiment 2—Hoppert-Webber-Canniff ration											
Control	17	Maxilla	0.8	0.3		0.8	0.3		1.7+	0.8+	
		Mandible	3.4	0.4	1.2	5.2	0.9	1.3	17.1+	3.1+	1.7
		Both	4.2	0.6		6.0	1.1		18.8+	3.5+	
Parotid duct tied and severed	17	Maxilla	1.4	0.3		1.4	0.3		3.1+	0.8+	
		Mandible	3.8	0.3	1.7	7.0	0.9	1.5	20.2+	3.1+	0.9
		Both	5.2	0.6		8.4	1.2		23.3+	3.8+	
Parotid gland extirpation	17	Maxilla	0.6	0.2		0.6	0.2		1.2+	0.4+	
		Mandible	3.4	0.3	0.3	5.8	0.8	1.3	16.6+	3.0+	1.1
		Both	4.0	0.4		6.4	1.0		17.8+	3.4+	

*SEM = Standard error of mean.

†CR = Critical ratio. The critical ratio is the ratio of the difference between two means to the standard error of the difference between the means. Wherever the critical ratio is less than 2.0, the difference between the means is considered to be statistically insignificant; from 2.0 to 2.9, moderately significant; 3.0 or higher, highly significant. The crisscross lines in the above table connect pairs of data that are being compared for the critical ratio test of significance.

In the second experiment where the Hoppert-Webber-Canniff coarse-particle ration had been substituted for ration No. 700, no significant increment in caries was observed in either operated group; nor could any significant difference in caries incidence be demonstrated between the two groups of operated rats. Again an almost complete atrophy was observed in the parotid glands of the rats whose parotid ducts had been tied and severed.

There was no observable significant difference in the caries incidence between the rats in the unoperated group in Experiment 1 and those in the unoperated group in Experiment 2. In both experiments, the incidence of dental caries was considerably higher than had been expected for offspring of the Hunt-Hoppert caries-resistant strain. This observation has been confirmed and extended in other experiments in our laboratories.

At autopsy, the submaxillary and major sublingual glands were carefully dissected free and weighed on a torsion balance to determine whether any sizable increase in gland weight had occurred in those rats whose parotid gland ducts had been severed or whose parotid glands had been extirpated, when compared with control animals as measured by the ratio of the gland weight to body weight. In our 2 previous experiments, described in 1955, highly significant increases in the ratio of submaxillary gland weight to body weight were observed in rats of the Harvard caries-susceptible strain where the parotid gland had been removed.[10] In the present experiment, consistent but numerically small increases in the ratio of submaxillary gland weight to body weight were observed among the operated rats in both experiments (Table II). The critical ratios for these differences varied from 1.9, 2.1, and 2.2 (significance at the 5 per cent level) to 2.9 (almost significance at the 1 per cent level). In one of the experiments described in 1955, a highly significant increase in the ratio of major sublingual gland weight to body weight was observed, whereas the increase in the other experiment was significant at the 5 per cent level. In the present experiments, numerically small increases in the ratio of sublingual gland weight to body weight among the operated rats were observed. However, these increases were either insignificant or barely significant at the 5 per cent level. In summary, it appears that the influences of the operative procedures in the present experiments followed the same trend with respect to the influence on the weights of the remaining glands although the results were not as consistent nor as striking. It is interesting also to note in the control group fed Harvard ration No. 700 that the weights of the parotid gland, the submaxillary gland, and the major sublingual gland had somewhat lower ratios to body weight than did the rats in the control group fed the Hoppert-Webber-Canniff diet. No explanation for this observation is available at present.

About the time that the above data were being evaluated, Brewer, Muhler, and Fischer[14] reported that sialoadenectomy caused major increases in caries incidence in mandibular molars but had no significant influence upon caries incidence in maxillary molars. Their experiments were conducted with Sprague-Dawley rats from which the submaxillary, sublingual glands, and the ventrolateral portions of the parotid glands had been removed bilaterally. The rats

TABLE II

THE INFLUENCE OF PAROTID DUCT EXCISION AND PAROTID GLAND EXTIRPATION ON THE BODY WEIGHTS, GLAND WEIGHTS, AND RATIOS OF GLAND WEIGHT TO BODY WEIGHT

	GAIN IN BODY WEIGHT (GM.)		WEIGHT OF PAROTID GLANDS (MG.)			WEIGHT OF SUBMAXILLARY GLANDS (MG.)			WEIGHT OF SUBLINGUAL GLANDS (MG.)		
	♂	♀	AVG.	SEM*	CR†	AVG.	SEM*	CR†	AVG.	SEM*	CR†
Experiment 1—Ration No. 700											
Control	254	148	261.8	22.6		387.7	13.4		65.4	2.2	
			(107.9)	(9.3)		(159.5)	(5.5)	2.2	(27.9)	(0.9)	0.6
Parotid duct tied and severed	257	139	---	---		444.1	14.6		74.5	4.4	
						(182.6)	(6.0)	2.9	(31.6)	(1.8)	1.8
Parotid gland extirpation	245	154	---	---		430.2	25.4		67.3	3.0	
						(186.6)	(11.0)	0.3	(28.9)	(1.3)	1.2
					1.8			2.1			2.1
Experiment 2—Hoppert-Webber-Canniff ration											
Control	218	126	287.2	20.9		394.4	14.2		69.2	2.6	
			(131.6)	(9.6)		(177.5)	(6.7)	1.9	(31.1)	(1.2)	1.2
Parotid duct tied and severed	215	121	---	---		409.9	10.9		74.8	3.2	
						(191.1)	(5.1)	2.5	(35.1)	(1.5)	2.1
Parotid gland extirpation	222	132	---	---		421.6	13.1		73.1	3.3	
						(189.2)	(5.9)	0.3	(33.4)	(1.5)	0.8

Values within parentheses are the ratios of glands to body weight in milligrams per 100 Gm.

*SEM = Standard error of the mean.

†CR = Critical ratio.

TABLE III

THE RELATIVE INFLUENCE OF SELECTIVE SIALOADENECTOMY ON THE DENTAL CARIES INCIDENCE IN MAXILLARY AND MANDIBULAR MOLARS

GLANDS REMOVED	JAW SCORED	1955—EXPERIMENT 1				1955—EXPERIMENT 2			
		NO. OF RATS	AVERAGE NUMBER OF CARIOUS TEETH	AVERAGE NUMBER OF CARIOUS LESIONS	AVERAGE EXTENT OF CARIOUS LESIONS	NO. OF RATS	AVERAGE NUMBER OF CARIOUS TEETH	AVERAGE NUMBER OF CARIOUS LESIONS	AVERAGE EXTENT OF CARIOUS LESIONS
Control	Maxilla	8	0	0	0	12	0.2	0.2	0.3+
	Mandible		0.6	0.8	1.0+		1.4	1.8	4.5+
Sham control	Maxilla	8	0.1	0.3	0.3+	--	--	--	--
	Mandible		0.5	0.5	1.0+		--	--	--
Extraorbital lacrimals	Maxilla	8	0.9	0.9	1.1+	11	0	0	0
	Mandible		1.1	1.1	1.8+		1.4	1.5	3.5+
Sialoadenectomy on left side	Maxilla	7	1.6	1.9	4.6+	11	1.6	2.4	5.6+
	Mandible		2.4	4.4	10.9+		3.5	5.3	13.2+
Complete sialoadenectomy	Maxilla	8	4.9	10.5	42.0+	10	5.7	11.2	39.8+
	Mandible		5.5	13.4	51.0+		5.9	14.4	50.0+
Parotids	Maxilla	7	3.7	5.7	15.9+	11	2.6	3.7	9.8+
	Mandible		4.6	7.0	19.0+		4.0	6.4	16.7+
Sublinguals	Maxilla	8	0.5	0.5	0.8+	10	1.3	1.6	3.0+
	Mandible		1.0	1.0	2.0+		3.1	4.0	8.5+
Submaxillaries	Maxilla	8	1.6	1.6	2.8+	9	1.8	2.6	5.3+
	Mandible		2.9	4.0	9.9+		4.2	6.8	18.9+
Parotids and submaxillaries	Maxilla	6	5.0	7.7	25.2+	11	3.1	4.7	14.5+
	Mandible		4.7	9.0	27.2+		3.9	7.4	21.8+
Sublinguals and submaxillaries	Maxilla	6	2.2	3.7	8.0+	10	2.3	3.8	7.2+
	Mandible		3.2	5.5	13.5+		3.6	6.3	16.5+
Parotids and sublinguals	Maxilla	7	2.3	3.3	7.4+	10	3.1	5.3	16.8+
	Mandible		3.1	5.0	12.1+		3.5	6.3	23.6+

had been maintained for 140 days on a cariogenic stock diet, the formula of which was a slight modification of the Hoppert-Webber-Canniff diet.

In view of the above report, the average caries incidence was determined for the maxillary molars and for the mandibular molars of the rats in Experiments 1 and 2 of the present series. These data as presented in Table I clearly indicated that sialoadenectomy under the conditions in Experiment 1 caused just about as great an increase in dental caries incidence in the maxillary molars as in the mandibular molars. Thus the data from the present experiments are in direct contrast to those of Brewer and associates[14] with respect to the influence of the removal of the major salivary glands on the maintenance of the maxillary molar teeth.

In the compilation of the data from the selective sialoadenectomy experiments described by Schwartz and Shaw,[10] only total influences of the operative procedures on the 12 molars were evaluated. Hence these data have been reconsidered to determine the relative influence of the removal of single pairs or combinations of pairs of salivary glands on the incidence of dental caries in maxillary versus mandibular molars. These evaluations are presented in Table III. Wherever the removal of a pair of salivary glands or a combination of pairs of salivary glands caused an increase in the total dental caries incidence, there was a substantial increase in caries incidence in both the maxillary molars and the mandibular molars. The mandibular molars consistently had a somewhat higher incidence of tooth decay than the maxillary molars. This finding is consistent with the numerous observations for intact rats where the mandibular molars routinely have a higher caries incidence than the maxillary molars. The only operated groups where the mandibular molars appeared to have been appreciably more adversely affected than the maxillary molars were those groups from which the submaxillary glands had been removed.

DISCUSSION

In earlier experiments by Schwartz and Shaw[10] with albino rats of the Sprague-Dawley strain that were maintained on refined cariogenic diet No. 700, surgical removal of pairs of the major parotid glands caused major increases in the occurrence of dental caries. In these studies, the evaluation of the influence of parotidectomy was made on the basis of a thorough inspection of the teeth after death by the procedure of Shaw, Schweigert, McIntire, Elvehjem, and Phillips.[13] However, Keller and co-workers[11] reported that no decrease in the average caries time occurred when the parotid duct and parts of the parotid gland were removed from rats of the Hunt-Hoppert caries-resistant and caries-susceptible strains. This observation was interpreted as an indication that interference with the parotid gland did not increase the caries susceptibility of rats of the Hunt-Hoppert strains. The rats in the latter experiment were maintained on the Hoppert-Webber-Canniff coarse-particle diet. The evaluation of tooth decay was based on an in vivo inspection of the rats to determine the age in days at which grossly visible carious lesions could be detected. In view of the distinct

differences in methods and the contrasting results, it appeared desirable to perform similar studies in our laboratories on the Hunt-Hoppert rats to attempt to resolve the contradictory results.

On the basis of our data, extirpation of the parotid gland or resection of the parotid duct did not cause appreciable dental caries increases when the Hunt-Hoppert caries-resistant strains were fed the Hoppert-Webber-Canniff diet until the first grossly visible carious lesions could be observed. However, major increases in dental caries incidence did occur when these rats were fed cariogenic diet No. 700 for the same time interval. While these observations do not explain why the removal of the parotid glands failed to cause an increased dental caries incidence, they do suggest that the results obtained by Keller and co-workers[11] cannot be explained on the basis of any unique, inherent characteristic of the Hunt-Hoppert caries-resistant strain. The difference between the results of Keller[11] and those of other workers can be explained to some extent on the basis of the different diets used.

It would be valuable to know whether a longer experimental period on the Hoppert-Webber-Canniff diet would have resulted eventually in an increased dental caries incidence among the operated groups of rats. In view of the definite increase in dental caries incidence that has been reported by other investigators in rats maintained on various natural diets after removal of one or more pairs of salivary glands, it seems reasonable to postulate that there might have been an increased dental caries incidence among the operated rats from the Hunt-Hoppert caries-resistant strain if the experimental period had been extended appreciably beyond the time when the first grossly visible lesions were observed. Even so, it seems strange that a trend in this direction would not have been established by the end of a 115-day experimental period, especially in view of the fact that the incidence of dental caries for the control rats fed ration No. 700 was approximately the same as that for control rats fed the Hoppert-Webber-Canniff diet.

Our data on the relative effect of sialoadenectomy on the incidence of carious lesions in maxillary and mandibular teeth are in contrast to the observations of Brewer and associates.[14] In all situations in our laboratories where the influence of salivary gland removal was tested with respect to the causation of dental caries in maxillary versus mandibular molars, the increased incidence of dental caries in all cases was almost as great in the maxillary teeth as in the mandibular teeth. In contrast Brewer and colleagues[14] had reported no increased incidence in maxillary teeth after sialoadenectomy. The experimental subjects in the latter study had been maintained on a cariogenic stock diet that was almost identical with the Hoppert-Webber-Canniff diet. The only apparent major difference between the experiment by Brewer and co-workers and our investigation was the composition of the diet and possibly the strain of rat. This difference is not likely to be explained on the basis of genetic variations, since our 1955 experiments were conducted with Sprague-Dawley rats as was the experiment by Brewer, and since our data from the Hunt-Hoppert caries-resistant strain and the Sprague-Dawley strain

were entirely comparable. On the basis of present information it would appear that the divergent results possibly may be explained in part by the difference in dietary composition between the 2 series of investigations.

Thus in 2 areas in which contrasting results have been observed between our studies on sialoadenectomy and those of other investigators, procedural differences exist which may explain the contrasting results. Since dietary differences were present in both cases, the question must be raised as to why these diets are responsible for variations in results, whether carious lesions of somewhat different etiology have resulted from the two dietary regimens, and which of the diets produce carious lesions that are the most similar to those in man. These divergent results demonstrate the need for clear recognition of all the factors involved in any experimental program and the continued need to refine assay procedures in order that false positive, false negative, or irrelevant observations may be eliminated. The question must be raised again as to whether the coarse-particle effect of the Hoppert-Webber-Canniff diet is undesirable and should be avoided in the production of experimental caries.

CONCLUSION

No significant increases in caries activity were observed in Hunt-Hoppert caries-resistant rats maintained for 115 days on the Hoppert-Webber-Canniff diet after resection of the parotid ducts or surgical removal of the parotid glands.

Highly significant increases in caries activity were observed among comparable rats fed high-sucrose, cariogenic diet No. 700 for the same period after parotid duct resection or parotid gland extirpation.

The increased caries activity among operated rats was as striking in the maxillary molars as in the mandibular molars. The latter observation has been corroborated by an evaluation of earlier data in which the removal of single pairs or combinations of pairs of salivary glands caused major increases in caries incidence in both maxillary and mandibular molars.

The current studies indicated that interference with the parotid gland caused consistent increases in the ratio of the submaxillary gland weight to body weight and smaller increases in the ratio of the major sublingual gland weight to body weight. The ratios of the parotid gland weight, submaxillary gland weight, and major sublingual gland weight were slightly higher for rats fed the Hoppert-Webber-Canniff diet than for those fed ration No. 700.

We are indebted to Dr. Lyon P. Strean, Merck, Sharp & Dohme Research Laboratories, West Point, Pa., for ample supplies of vitamin B complex.

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THE CURATIVE ACTION OF α TOCOPHEROL AND OF PROTEIN UPON THE INCISOR TEETH OF VITAMIN E-DEPLETED RATS

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THE histologic changes in the enamel organ of the rat's tooth during vitamin E deficiency have been described.[6, 10, 12, 13] The present paper describes the histologic picture produced by the curative action of α tocopherol in the teeth of rats depleted in vitamin E and the effect of additional protein in the diet of vitamin E-depleted rats.

EXPERIMENTAL

Diets.—The same vitamin E-free diet was used as described previously,[10] and consisted of potato starch, dried brewer's yeast, and cod liver oil. The levels of cod liver oil were either 20 or 2.5 per cent, the content of potato starch being adjusted accordingly. Since the same histologic results were obtained with both diets, they will be referred to collectively as Diet 1. The animals grew much better on the diet with the lower cod liver oil content (protein content, 9.3 per cent). Diet 2 contained 2.5 per cent cod liver oil and enough egg albumin to raise the protein content to 18.8 per cent, the amount of starch being correspondingly reduced. When α tocopherol was given, 3 mg. of α tocopheryl acetate (Ephynal*) was administered daily in 3 Gm. of freshly made diet.

Rats.—Albino rats of the Wistar Institute strain were employed. Both sexes were used indiscriminately, as similar results were obtained with each. The rats were placed on Diet 1 when they weighed about 50 grams. Thirty or 40 days later they were divided into 3 groups.

Group 1. (66 rats): Some rats were sacrificed at this time and others were continued on Diet 1 and killed at intervals up to 120 days.

Group 2. (60 rats): These animals were now given 3 mg. of α tocopherol daily and were sacrificed at intervals from 10 to 80 days after the change of diet.

Group 3. (30 rats): These animals were transferred to Diet 2 and were killed at intervals after being on this diet for 40 to 80 days.

A fourth group of rats were positive controls and received 3 mg. of α tocopheryl acetate daily with Diet 1 from the beginning of the experiment for up to 40 days.

The groups of animals killed at each stage varied in number between 4 and 18 (192 rats in all were used). The plan of the experiment is summarized in Table I.

Received for publication Dec. 28, 1957.

*Roche Products, Ltd., Johannesburg, S. A.

TABLE I

TREATMENT OF RATS AND TIMES OF KILLING AFTER THE EXPERIMENT BEGAN

GROUP	NUMBER IN GROUP	DAYS ON DIET 1 (VITAMIN E-FREE)	DAYS ON DIET 1 PLUS 3 MG. α TOCOPHEROL DAILY, AFTER DIET 1 ALONE	DAYS ON DIET 2 (ENRICHED IN PROTEIN) AFTER DIET 1	DAYS ON DIET 1 + 3 MG. α TOCOPHEROL FROM START OF EXPERIMENT
1.	66	30, 40, 70, 80, 90, 100, 110, 120	--	--	--
2.	60	30 or 40	10, 20, 30, 40, 60, 80	--	--
3.	30	30 or 40	--	40, 60, 80	--
4.	36	--	--	--	20, 30, 40

The color of the upper incisor teeth was noted at intervals during the experiment and also at death; blood was taken for the dialuric acid hemolysis test,[3] and the skulls were decalcified and embedded for longitudinal section of the upper incisor teeth. One tooth, after formalin fixation, was stained with hematoxylin and eosin. The other tooth, after fixation in Bouin's solution, was decalcified with 10 per cent acetic acid and stained for iron with ferrocyanide and HCl. This procedure was found to be the best for demonstrating the iron content of the ameloblasts.

RESULTS

Group 1.—All animals showed the typical degenerative change in the enamel organ (Fig. 1). All the teeth were white by 30 days and the dialuric acid test was positive in all rats. In normal rats, the ameloblasts on the incisal side of the tall ameloblasts contain yellow translucent granules which are very rich in iron and which are probably the precursors of the yellow pigment on the incisor tooth.[9] In vitamin E deficiency, the number of these granules, as evidenced by the iron content of the ameloblasts, was progressively reduced, and by 30 days these cells were iron free or had but traces only.

Group 2.—The results were the same if the preliminary depletion period was 30 or 40 days. At the end of that time the teeth of all rats were quite white. The color was regained after the supplementation of the diet with α tocopherol was begun. The first appearance of the yellow pigment at the gingival margin was between 29 and 64 days later, averaging, in the whole group, 42 days.

The first sign of recovery, which was seen before any structural alteration in the enamel organ, was the reappearance of iron in the ameloblasts, which had occurred by 10 days after dosage was started. In the early stages there were too few iron-containing cells but, as the enamel organ recovered, more iron-containing ameloblasts appeared. At the same time, the macrophages, which during vitamin E deficiency are very abundant in the fibrous tissue which replaces the enamel organ and which contain large amounts of iron rich pigment, became more and more reduced in number as the experiment proceeded.

No detectable improvement was seen in sections stained with hematoxylin and eosin until 40 days of α tocopherol therapy, during which period the tooth

had largely replaced itself. The outer enamel epithelium recovered first and gradually extended forward until it reached and blended with the gingival epithelium. This was seen in a few rats at 40 days. The ameloblasts regressed far too soon at this stage, and the outer enamel epithelium distally was covered by a very flattened layer of cells (Fig. 2). In later stages, ameloblasts extended forward as far as the gingival crest of alveolar bone, but they were low cubical and not columnar cells. The ameloblasts had not recovered completely by 80 days. In most cases, when the teeth had not recovered their color, fibrous tissue

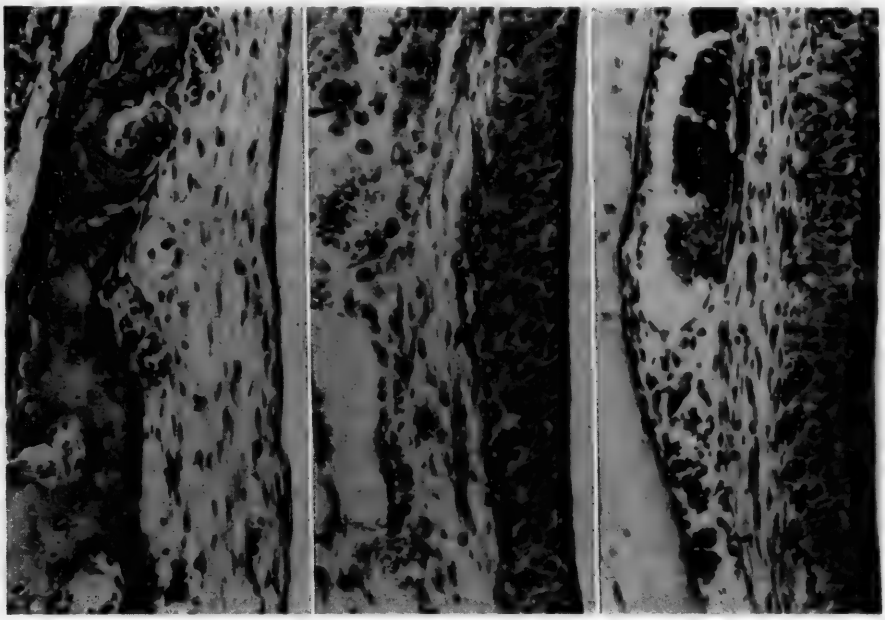

Fig. 1. Fig. 2. Fig. 3.

Figs. 1-3.—All photomicrographs are longitudinal sections of the enamel organ toward the incisal end of the upper incisor tooth of the rat. (Orig. mag. ×120.)

Fig. 1.—Rat 286 on the vitamin E-free diet for 30 days, showing replacement of the enamel organ by fibrous tissue.

Fig. 2.—Rat 145 on the vitamin E-free diet for 30 days and then given 3 mg. of α tocopherol daily for a further 40 days. The outer enamel epithelium has recovered but the ameloblasts are a flattened layer.

Fig. 3.—Rat 387 on the vitamin E-free diet for 40 days and then given additional protein in the diet for a further 60 days. The outer enamel epithelium has recovered but the ameloblasts are flattened cells. The same was seen exactly in the α tocopherol-dosed rats at this stage.

still replaced the enamel organ at the incisal end of the tooth. When the outer enamel epithelium extended to the end of the tooth, macrophages disappeared except at the gingival margin, where a few are always normally present.

The dialuric acid test was faintly positive (at 24 hours) after 10 days of α tocopherol therapy, but was consistently negative after that.

Group 3.—The results with these animals were virtually the same as with those of Group 2, and the length of the preliminary depletion period made no difference. At the end of the depletion period, all teeth were white. The yellow

pigment reappeared at the gingival margin 29 to 64 days after dosing began, the average being 47 days. The subsequent sequence of histologic changes was exactly the same as seen in the α tocopherol-treated rats (Fig. 3), and iron-containing ameloblasts reappeared in exactly the same manner. The dialuric acid test was, however, positive throughout.

Group 4.—These animals, on the vitamin E-free diet plus 3 mg. α tocopherol daily from the beginning of the experiment, had teeth of orange-yellow color throughout and their enamel organs were normal histologically. All dialuric acid tests were negative.

DISCUSSION

Granados, Mason, and Dam[2] found that normal pigment was redeposited on the incisor teeth of vitamin E-depleted rats some weeks after giving α tocopherol, and Moore,[11] in similar experiments, found restoration of tooth color in from 4 to 7 weeks, figures which agree with those reported here. But, since these color changes are only seen after the pigmented part of the tooth has erupted, it is obvious that curative changes had started some time earlier, and the present results show that by 10 days the iron content of the ameloblasts began to be restored. This is similar to the speed of curative action of vitamins D[7] and A[15] upon teeth in rickets and avitaminosis A. The dialuric acid hemolysis test, likewise, began to be negative at about this time.

It is, however, difficult to see why the replacement of the degenerated enamel organ took so long. It would seem to indicate that, in general, the bodily needs for α tocopherol after depletion must be high and that only small amounts can be "spared" for the tooth, although large amounts of α tocopherol were given. It is also interesting to see how the enamel organ recovered in stages, the outer enamel epithelium being normal at the incisal end of the tooth when the ameloblasts were either flattened or still too small. Irving[8] has suggested that the outer enamel epithelium has an organizing influence on the ameloblasts and that their recovery from degenerative changes depends on an intact outer enamel epithelium. This would explain, in part, the sequence of events observed here.

It may be questioned whether the ameloblasts in the anterior half of the enamel organ have any function at all, since teeth normal in color and structure were produced when the incisal end of the enamel organ had virtually no recognizable ameloblasts. It seems that once the ameloblasts have laid down, matured, and pigmented the enamel, their functional life is finished and, from the work of Hoffman,[4] it would appear that their rate of regression depends solely on the rate of eruption of the tooth. Stein[16] and Schour[14] consider, as does the present writer,[8] that ameloblasts normally have a limited life span of work. It would seem that, as in the present experiments, their life span can be considerably curtailed without affecting their functional activity.

In the early stages of recovery with α tocopherol dosage, when the tooth was still white, it was found that ameloblasts deposited the pigment on the tooth but that macrophages, present in the fibrous tissue that had replaced the enamel organ more anteriorly, took this pigment off again.[9] The outer enamel

epithelium exerted a protective action on the tooth pigment, since once these layers of the enamel organ extended to the end of the tooth, the macrophages disappeared and the color in the erupted part of the tooth was preserved.

Several workers have shown that protein has a partial protective action on the pigmentation of the incisors of rats on vitamin E-free diets.[5, 11] In the present experiments, although extra dietary protein did not reverse the dialuric acid test, the tooth recovery, both in color and histology, was as good as when α tocopherol was administered. Protein is apparently the only dietary constituent which has this α tocopherol-like action on teeth which is not shared even by dietary iron.[1]

SUMMARY

1. Young rats were maintained on a vitamin E-free low-protein diet for 30 or 40 days, by which time all their upper incisor teeth were white. They were then divided into 3 groups.

2. The enamel organs of the group kept on the diet for up to 80 days showed marked degeneration and the iron-containing granules of the ameloblasts had disappeared by 30 days. All had positive dialuric acid hemolysis tests.

3. A second group was maintained on the vitamin E-free diet but was given 3 mg. of α tocopherol daily for up to 80 days. The dialuric acid tests became negative after 10 days and the incisal pigment reappeared on an average of 42 days later. Iron was redeposited in the ameloblasts 10 days after dosage was started, but the morphologic recovery of the enamel organ was much slower. The outer enamel epithelium recovered first and even by 80 days the ameloblasts at the incisal end of the tooth were not completely normal.

4. A third group of rats were transferred to a vitamin E-free diet with a raised protein content for up to 80 days. The dialuric acid test remained positive throughout, but incisal pigment reappeared on an average of 47 days after the change of diet. The recovery of the enamel organ was exactly the same as in the group of rats dosed with α tocopherol. Under these experimental conditions, extra dietary protein was just as adequate as α tocopherol as a curative agent for the teeth.

5. It is suggested that the outer enamel epithelium has an organizing effect on the ameloblasts and is essential for their recovery from various insults. The ameloblasts at the incisal end of the tooth appear to have no function, since normal teeth can be produced in their absence.

The author is indebted to Dr. C. C. Solomons for carrying out the dialuric acid hemolysis tests, and to Roche Products, Ltd., for gifts of Ephynal.

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EXPERIMENTAL STUDIES ON CALCIFICATION OF TOOTH GERMS IN VITRO

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IN the last few years there has been a renewed interest in the calcification of bones. From the results obtained on the study of cartilage and bone it has been assumed that the calcifying mechanism in teeth is similar. Probably there is a close relationship, but the morphology of bone and cartilage is very different from that of teeth. Calcification, in vitro, as described by Robison and Rosenheim,[13] has been studied by a number of workers, but only Fell and Robison[2, 3] (avian bones) and Niven and Robison[11] (long bones of rabbits) used normal tissue; other workers used rachitic bones. Hirschman, Sobel, and Fankuchen[7] studied the x-ray diffraction pattern of bones calcified in vitro and found an apatite crystal structure as in normal bone. It was thought that a series of experiments on the calcification of teeth in vitro of normal animals would help to elucidate the mechanisms involved in this process.

NORMAL WHOLE TEETH

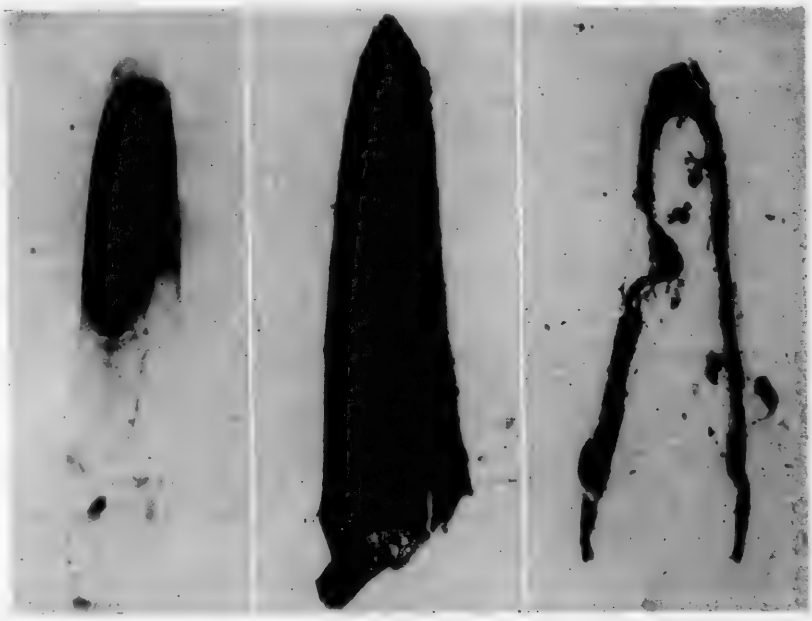
Material and Methods.—Incisor tooth germs of 19- to 21-day embryo rats and 1-day postfetal rats, 14- to 16-day hamster embryos, and 18- to 20-day embryo mice and 1-day postfetal mice were used. The tooth germs were dissected from upper and lower jaws with a cataract knife and a needle under a dissecting binocular microscope. They were then washed in normal saline before being transferred to the calcifying solutions. One tooth from one side of each jaw served to demonstrate the calcification already present, while the other tooth was used for the experiments to see how far calcification could proceed in the various solutions. The solutions used were similar to those employed for the calcification of rachitic cartilage.[13] They are referred to in this work by figures representing the concentration of calcium, inorganic and organic phosphate in milligrams per 100 ml. The solution, 8.5.10, contained 8 mg. Ca, 5 mg. phosphorus as inorganic phosphate, and 10 mg. phosphorus as organic phosphate per 100 ml. basal salt solution, and the solution 8.5.0 contained no organic phosphate; the organic phosphate used was sodium β-glycerophosphoric ester. The *basal salt solution* (grams per liter) was NaCl, 6.0; $NaHCO_3$, 2.2; KCl, 0.3; and $MgSO_4(7H_2O)$, 0.25. The pH of the solutions was 7.4 to 7.9 and the period of incubation was 18 to 24 hours at 37° C. The control teeth were immersed in normal saline and incubated at the same time.

The study on which this paper is based was supported by a grant from The Medical Research Council.

Received for publication Jan. 1, 1958; revised by author Feb. 3, 1958.

After incubation, the tissues were washed, fixed in 10 per cent neutral formalin for ½ hour, washed again and left in 2 per cent silver nitrate for 12 to 24 hours, then dehydrated in acetone. The teeth were sectioned and counterstained with safranine.

The control sections showed that in most of the tooth germs there was a layer of dentin, and in the younger ones there was just a trace of calcification. The older teeth had a layer of enamel and dentin, some of which was calcified. In all the teeth there was an area of uncalcified dentin, or uncalcified enamel and dentin. Osteodentin is always present in the tip of the pulp of the persistent growing rodent incisor and is covered by a thin layer of dentin. The osteodentin, except in a few instances, was not calcified in the tooth germs used in these experiments.



A. *B.* *C.*

Fig. 1.—*A*, Whole mount of control cap, placed in normal saline, showing uncalcified areas. *B*, Whole mount of cap immersed in 8.5.10 solution. Note that the whole cap is calcified. *C*, Section of a whole cap after immersion in 8.5.10 solution. Note the absence of cells.

Observations.—In the teeth incubated in the calcifying solutions, the calcified dentin and enamel extended beyond the original areas, toward the base of the tooth. The osteodentin always calcified in the 8.5.10 solution, but often remained uncalcified in some of the teeth in the 8.5.0 solution. In general, the calcification was less complete in teeth incubated in the solution containing no organic phosphate.

The use of the whole tooth germ had two disadvantages: (1) poor penetration of the solutions through the cellular tissue caused incomplete calcification, and (2) the cells became wholly necrotic. In order to eliminate these

disadvantages, attempts were made to conduct experiments on the isolated formed matrix, free of the surrounding cells. This method has several advantages over that of using the whole tooth. In the first place, the formed matrix is in contact with the solution. Moreover, for the first time it is possible to study a calcifiable material in the absence of cells.

Cap Method for Normal Teeth.—Whole incisors were dissected from the jaw and the enamel organ stripped off. This exposed a cap of dentin which, according to the age of the tooth, might include some enamel. The pulp was then expelled from the cap by gentle pressure with a cataract knife and, as far as possible, cellular tissue was scraped away (Fig. 1, *A*). Sometimes the cap was cut open, laid flat, and the cells scraped from it. It was not always possible to remove every cell, but the very few remaining cells, when present, were considered to have no influence on the experiment. In the caps which were used for these experiments, the tips were partially calcified, except for the osteodentin. In the very young teeth in which there was no calcification, difficulty was experienced in removing the matrix from the soft tissues.

Sometimes the calcified apical portion of the cap was cut off, leaving the lower uncalcified matrix, free of cells, for the experiment (Figs. 2, *A;* 3, *A*). The whole caps and the portions of uncalcified matrix were placed in 8.5.10 and 8.5.0 solutions and the controls in normal saline; both sets were incubated at 37° C. for 18 to 24 hours.

Observations.—Whole caps and uncalcified portions of enamel and dentin matrix calcified completely when incubated in the 8.5.10 solutions. Calcification was less complete in the 8.5.0 solution (Figs. 1, *B;* 1, *C;* 2, *B;* 3, *B*).

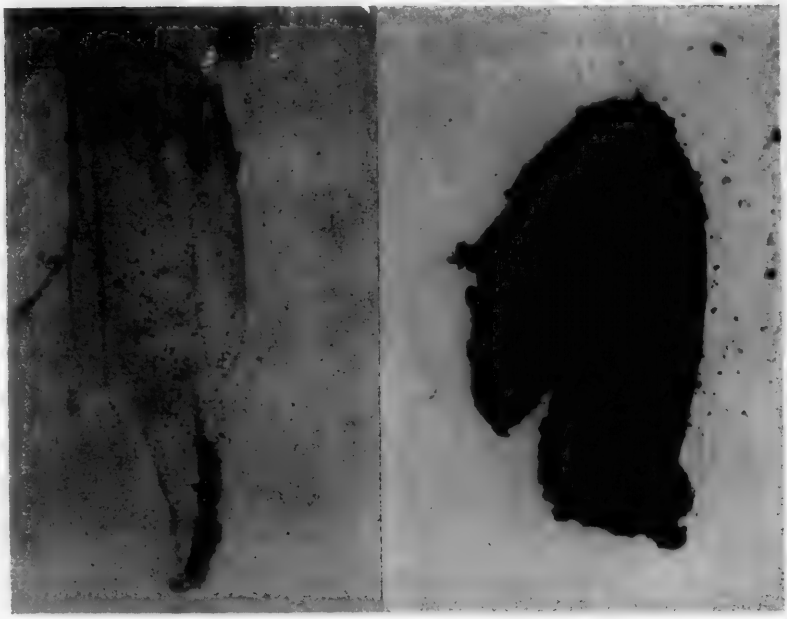
EXPERIMENTS WITH BERYLLIUM

Sobel, Goldfarb, and Kramer[16] and Sobel[14] found that the cartilage of rats fed on a beryllium diet would only calcify very imperfectly when incubated in calcifying solutions, whereas bones of animals fed on a calcium deficient diet calcified completely in a similar solution.

It has been shown by Irving[8] and by Wentz[18] that beryllium affected the development of teeth in vivo. Irving injected beryllium salts into rats with resultant deposition of abnormal dentin; the enamel was not affected. Wentz, however, fed rats on a beryllium diet and found that both enamel and dentin were poorly calcified.

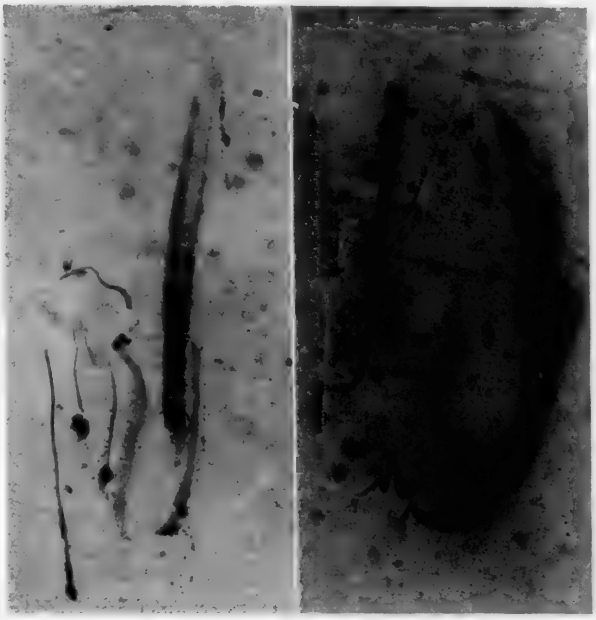
Gutman and Yü[5] fed rats on a "Schneider-Steinbok rachitogenic diet." Bone slices from these rats were incubated in calcifying solutions containing only organic phosphates. Beryllium was found to inhibit calcification to a marked degree. A similar set of experiments have been made by me on tooth germs from normal animals.

Material and Methods.—Caps of dentin and enamel were placed in solutions 8.0.10 with sodium β-glycerophosphate as the sole source of phosphate. To this solution beryllium sulfate was added in amounts sufficient to give the



A. *B.*

Fig. 2.—*A*, Control whole mount of piece of formed matrix of dentin and enamel placed in normal saline. Note all tissue is uncalcified. *B*, Whole mount of piece of formed matrix immersed in 8.5.10 solution. Note the absence of cells.



A. *B.*

Fig. 3.—*A*, Control section of formed matrix placed in normal saline. *B*, Section of formed matrix after immersion in 8.5.10 solution. Note the absence of cells in both sections.

final molarities of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}. Control caps were placed in 8.0.10 solution and in normal saline; all sets were incubated at 37° C. for 24 hours (Fig. 4).

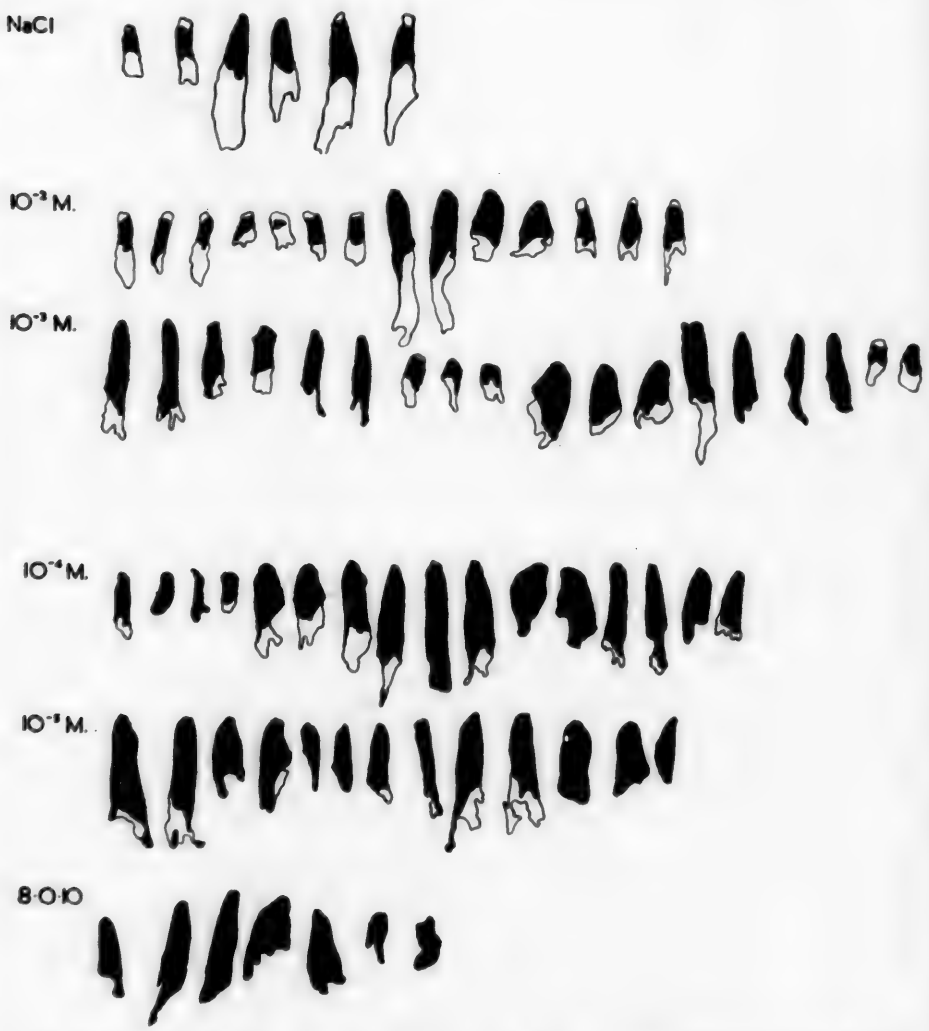


Fig. 4.—Drawings of whole caps immersed in normal saline only, in solutions of 8.0.10 to which beryllium was added, and in 8.0.10 solution.

Observations.—In the caps inserted in the 8.0.10 solution alone there was complete calcification. In those incubated in the solution with beryllium salts there was partial or complete inhibition of calcification (Fig. 4). The fraction of caps in which there was some inhibition is as follows: 10^{-2}M—1.0, 10^{-3}M—0.84, 10^{-4}M—0.52, and 10^{-5}M—0.46.

The results with caps, from normal animals, immersed in beryllium correspond with those of Gutman and Yü in a similar experiment with rachitic bones.

EXPERIMENTS WITH PROTAMINE

Protamine was first discovered by Miescher, in combination with nucleic acid, in the sperm head of the salmon (Greenstein[4]). Sobel[15] made use of the strongly basic properties of protamine to study the role of acid substances in cartilage matrix, by allowing calcium ions and protamine to compete for the matrix, in suitable mixtures. He first immersed rachitic bones in a basal salt solution to which protamine was added. These bones were then placed in a solution which would calcify rachitic bones and, thus, he observed that the protamine-treated bones failed to calcify.

A. *B.*

Fig. 5.—*A*, Control whole mount of cap placed in normal saline. *B*, Whole mount of cap after immersion in 8.5.10 solution to which protamine was added. Note some inhibition of calcification of the matrix in the lower portion of the cap.

A set of experiments were made in which caps of dentin and enamel from normal animals were incubated in calcifying solutions to which protamine was added.

Material and Methods.—Caps of dentin and enamel were removed from incisor teeth and immersed in calcifying solution 8.5.10, containing 1 to 4 per cent protamine sulfate. Control caps were placed in 8.5.10 solution alone, and in normal saline; all sets were incubated at 37° C. for 24 hours (Figs. 5, *A*; 6, *A*).

Observations.—In some of the caps, calcification extended a little beyond the original area; nevertheless, in most of them calcification was inhibited. It was noticeable that the osteodentin, with its thin covering of dentin, failed to calcify completely even in the solution 8.5.10 when protamine was present (Figs. 5, *B;* 6, *B;* 6, *C*). Also the predentin, adjacent to the calcified dentin, which was present at the time of the experiment, remained uncalcified, as well as the matrix near the base of the tooth. The solution containing as little as 1 per cent protamine affected calcification, but maximum inhibition was obtained with the 3 per cent protamine.

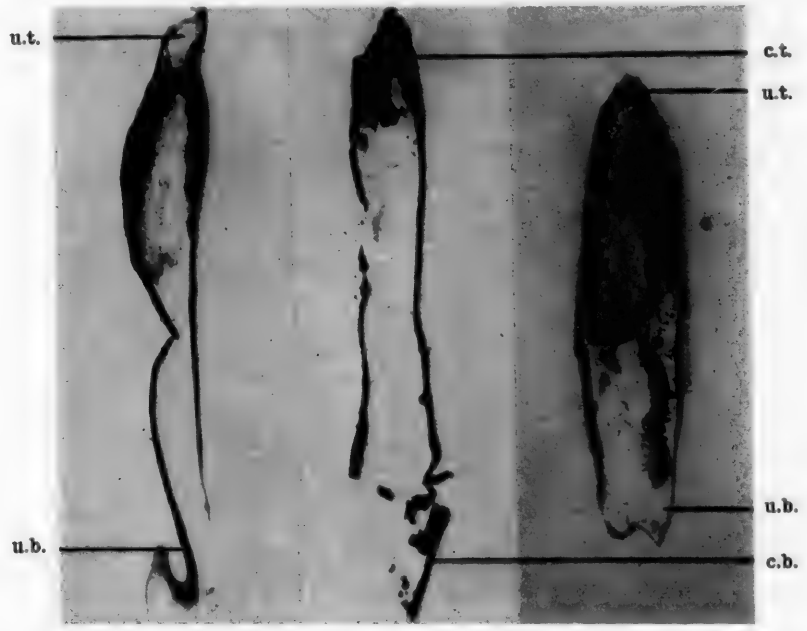


Fig. 6.—*A*, Control section of cap placed in normal saline. Note the uncalcified areas at tip and base of tooth. *B*, Section of cap immersed in 8.5.10 solution. Note complete calcification. *C*, Section of cap immersed in 8.5.10 solution to which protamine was added. Note the inhibition of calcification at the tip and at the base of the tooth. *c.b.*—Calcified base; *c.t.*—calcified tip; *u.b.*—uncalcified base; and *u.t.*—uncalcified tip.

EXPERIMENTS WITH TESTICULAR HYALURONIDASE

Certain experiments on rachitic bone slices have suggested that there is a substance in the matrix which accepts the calcium ions. Sobel[15] found that rachitic bone slices treated with strontium chloride, before immersion in a calcifying solution, failed to calcify. From this work he concluded that strontium combines with "a factor in the matrix essential for calcification." One of the significant factors in calcification is the combination of calcium with some substance or substances in the ground substance.

Pincus,[12] and Hess and Lee[6] identified an acid mucopolysaccharide, chondroitin sulfate, in dentin matrix. Leblond, Belanger, and Greulich[10] and

Belanger[1] described experiments in which S^{35} was injected into rats. Autoradiographs were then made of the teeth which showed the S^{35} located in the dentin matrix. Sections of dentin showed no autoradiographic reaction after digestion with hyaluronidase. From this result it was assumed that the matrix, which was hydrolyzed by the enzyme, was chondroitin sulfate.

Calcification Experiments.—These observations were followed up by calcifying experiments on dentin caps digested with hyaluronidase.

Material and Methods.—Some freshly dissected caps of dentin and enamel that had been digested with testicular hyaluronidase for a period of 4 hours were immersed in the calcifying solution 8.5.10. Control, undigested, caps were placed in 8.5.10 solution alone and in normal saline; the 3 sets were incubated for 24 hours at 37° C.

Observations.—The caps digested with hyaluronidase and incubated in the calcifying solution 8.5.10 resulted in complete calcification comparable to the undigested ones in a similar solution.

METACHROMASIA IN THE DEVELOPING TOOTH

A histologic study for metachromasia was made of caps used in the calcifying experiments. Immediately after dissection some caps were digested with hyaluronidase. Both digested caps and undigested caps were fixed in formalin, sectioned at 8 microns, and stained with toluidin blue at normal pH. The matrix of the caps digested with hyaluronidase showed no metachromasia, whereas the undigested matrix was metachromatic.

To obtain further evidence that a chondroitin sulfate was present in the predentin, metachromasia of the tissue used for calcifying experiments was studied by the methylene blue extinction method.

Whole teeth, and caps, as used for the previous experiments on calcification, were fixed in alcohol. Sections were cut at 8 microns and stained with buffered methylene blue at pH levels ranging from 1.7 to 5.9. At pH between 3.9 and 4.9, the predentin and dentin showed very faint metachromasia. These results indicate that the ground substance is not sufficiently metachromatic to suggest the presence of an acid mucopolysaccharide such as chondroitin sulfuric acid.

DISCUSSION

It has been shown that the enamel and dentin matrix in developing teeth calcifies when immersed in a suitable calcifying medium. Moreover, when the cellular tissue has been removed from the tooth germs the isolated matrix formed will calcify. From these latter results it can be concluded that the enzyme alkaline phosphatase present in the cells is not essential for the process of calcification. Histochemical tests for alkaline phosphatase did not reveal any enzyme in the formed matrix. The conclusions reached are that the enzyme is present at a time when there are great metabolic changes in the cells and when protein matrix is formed.

Evidence has been given by Klemperer, Miller, and Hill[9] that beryllium inhibits alkaline phosphatase. In the caps used for the experiments with beryllium, most of the cells in which the enzyme was present were removed; so, under these circumstances, the beryllium can hardly be acting as an enzyme inhibitor. It may be that the beryllium was competing with the calcium ions, so preventing their deposition, and a similar explanation might account for the inhibitory effect of protamine.

The substance removed by hyaluronidase from the ground substance of the dentin does not play a part in calcification. Belanger[1] claimed that hyaluronidase removed the chondroitin sulfate from the ground substance as no S^{35} was present in sections digested with the enzyme. It is possible that the radioactive sulfur indicates the presence of some sulfate other than chondroitin sulfate which is removed by hyaluronidase. Sognnaes[17] gave support to the view that the dentin matrix is not sufficiently basophilic for much chondroitin sulfate to be present. This is a suggestion in agreement with the results in this paper.

Although Hess and Lee[6] isolated chondroitin sulfate from the dentin, they suggested that improvements in the methods of analysis were necessary before the accurate estimation of the acid mucopolysaccharide could be made. If chondroitin sulfate is present in very small quantities in the dentin then it may be that the substance will not be identified by histochemical tests. Yet its presence, even in small amounts, is no indication that chondroitin sulfate plays an important part in the calcification of the dentin. There must be some special mechanism responsible for the observed deposition of calcium salts in the ground substance of dentin and enamel. These tissues calcify when exposed to a suitable calcifying medium, whereas other tissues under similar conditions will not calcify. Any concept of the factors responsible for calcification involves two main tissues, the cell and the matrix. However, in the experiments described above, one of the important constituents, the cell, was usually absent; yet despite this situation the matrix calcified. Further investigation of the calcifying mechanism in the absence of cells will clearly be of value.

SUMMARY

1. Whole tooth germs, caps, and formed matrix of rats, mice, and hamsters have been calcified in vitro.

2. In the caps and the formed matrix, cells in which alkaline phosphatase is present have been removed and yet the dentin and enamel matrix calcified, so intracellular alkaline phosphatase does not appear to play a part in calcification.

3. Beryllium and protamine added to the calcifying solutions inhibit calcification by competing with the calcium salts and preventing its deposition in the matrix.

4. Caps digested with testicular hyaluronidase calcified in the solutions.

5. Chondroitin sulfate does not play an important part in the calcification of dentin.

The author gratefully acknowledges the facilities placed at her disposal by Professor Boyd. She also wishes to thank Mr. R. A. Parker for his technical assistance and Mr. J. F. Crane for the preparation of the photographs.

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BRITISH DIVISION

OF THE

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

PROCEEDINGS OF THE SIXTH ANNUAL MEETING

APRIL 10TH AND 11TH, 1958

SUTHERLAND DENTAL SCHOOL, KING'S COLLEGE, NEWCASTLE-UPON-TYNE

Compiled by Ivor H. Kramer, Editor, British Division

1. PREPARATION OF ENAMEL POWDER IN WATER.—*C. P. Wallis, Department of Biochemistry and School of Dental Surgery, University of Edinburgh.* The Manly and Hodge separation (*J. D. Res.* 18: 133, 1939) has been modified to avoid drying the enamel. The crowns of extracted teeth are cleaned and ground under water in an electric mortar and pestle at 1° C., pouring off the suspension at 15-minute intervals. The powder is transferred to a 50 ml. centrifuge tube, washed with acetone, and centrifuged at 4,000 g for 15 minutes at 5° C. in bromoform/acetone of density 2.7. The dentine can be poured off with the liquid and each fraction is again centrifuged in the bromoform mixture. The enamel sediments are washed with acetone and water and stored at 0° C.

2. REMOVAL OF FLUORIDE FROM SOLUTIONS OF SODIUM AND STANNOUS FLUORIDE BY POWDERED ENAMEL.—*S. A. Leach, Turner Dental School, Manchester, F. A. Smith, D. E. Gardner, and H. C. Hodge, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.* Hatton, Nebergall, and Muhler (*J. D. Res.* 34: 350, 1955) reported that powdered enamel removed more fluoride from solutions of stannous fluoride than from solutions of sodium fluoride which initially contained stoichiometrically equivalent amounts of fluoride. Shaking powdered enamel in the fluoride solutions increased the pH, markedly for stannous fluoride and detectably for sodium fluoride. On increasing the pH of stannous fluoride solutions in the absence of enamel, precipitates were formed which contained sufficient fluoride that might account, at least in part, for the greater amount of fluoride removed from stannous fluoride solutions. The fluoride removed as precipitate from a series of stannous fluoride solutions on increasing the pH was measured and the fluoride removed at any arbitrarily chosen series of initial fluoride concentrations could be found by interpolation. From the data of Hatton and co-workers, in stannous fluoride solutions shaken with enamel, the fluoride similarly removed could be found at the same interpolated initial concentrations. No discrimination could be drawn between data from stannous and sodium fluoride solutions at the lower concentrations of fluoride. At the higher concentrations more fluoride may have been taken up from the stannous fluoride solutions; however, the differences are not great and the higher initial acidity may have been responsible.

3. The Rate of Uptake and Release of Radioactive Fluoride and Other Ions by the Hard Dental Tissues.—*J. H. Fremlin and J. L. Hardwick, Departments of Physics and Dental Surgery, University of Birmingham.* In vitro studies on teeth immersed in labelled 1 ppm sodium fluoride consistently show rapid uptake of F^{18} by intact enamel during the first few minutes with a subsequent gradual decrease to a slower but uniform rate for at least the next 24 hours; this later stage is accompanied by detectable penetration of the enamel. The rate of uptake is increased in solutions of lower pH and is greater in enamel from which the surface layers have been removed, and hypoplastic and young enamel. In carious lesions of the enamel and dentine, and in cementum, the rate of uptake is many times greater and is accompanied by more rapid penetration. The uptake of F^{18} is largely an exchange with the indigenous enamel fluoride. The rate of uptake of radioactive sodium by intact enamel is similar but less rapid; the sodium usually penetrates the full thickness of intact enamel in 14 to 28 days. After immersion in 1 ppm sodium fluoride solutions, labeled with F^{18}, Na^{22}, or Na^{24}, the release of the isotope concerned, from intact enamel by washing in running tap water (0.05 ppm of fluoride), is relatively rapid during the first few minutes but decreases to a slower but uniform rate subsequently; the release of F^{18} is slower than the uptake. From enamel surfaces similarly treated, the release of F^{18} during immersion in buffers at pH 4.6 is very rapid.

4. On the Occurrence of Whitlockite in Dental Calculus.—*S. L. Rowles, M.R.C. External Staff, Department of Dental Pathology, University of Birmingham.* Whitlockite, which has the same crystal structure as β $-Ca_3(PO_4)_2$, a high temperature form of calcium phosphate, has been found to occur very frequently in human dental calculus. The calculus whitlockite shows a characteristic displacement of the lines of its x-ray diffraction pattern compared with β $-Ca_3(PO_4)_2$. This is due to the presence of small amounts of magnesium which make the lattice stable at body temperature. Magnesium whitlockite has been prepared from aqueous solution at 37° C. in a well crystallized form by the action of water on the secondary calcium phosphate, brushite, in the presence of magnesium ions. A preliminary investigation of the conditions of formation and solubility properties of magnesium whitlockite has been carried out.

5. Some Observations on the Composition of Calculus.—*E. M. Westerden and K. Little (M.R.C. Staff), Nuffield Orthopaedic Centre Oxford.* X-ray diffraction is being used to analyze dental and salivary calculus. Compounds found in unheated samples are hydroxyapatite, octacalcium phosphate, brushite, monetite, calcite, and possibly another phase. In the presence of magnesium (Tovburg-Jensen and Rowles, *Acta odont. Scandinavica* 15: 121, 1957) whitlockite is found. After heating to 1,000° C., the main compounds found are hydroxyapatite and whitlockite, with possibly pyrophosphate and an unidentified compound. The amount of whitlockite in the heated specimens tends to reflect the Ca/P ratio. More variation is found in the composition of dental than salivary calculus. In both, different parts and layers of the same calculus can have different compositions. More octacalcium phosphate is found in dental than in salivary gland calculus, and more is found in the denser regions of the calculus. We find octacalcium phosphates in dental calculus more often than brushite. By contrast, Tovburg-Jensen and Gabhard Hansen (*Experientia* 13: 311, 1957) found brushite a more frequent constituent in Copenhagen. In a few cases, monetite is also observed. None of these compounds—or whitlockite—seem to

be mutually exclusive. The results suggest that all three theories of the precipitation of apatite (direct, through monetite, and through octacalcium phosphate) may hold under different conditions.

6. Observations on the Pattern of Mineralization of Human Dental Enamel.—*H. S. M. Crabb and A. I. Darling, Dental School, University of Bristol.* Microradiographs of ground sections and stained decalcified sections of developing teeth have been examined. In the pattern of mineralization of the enamel the following features were found: (a) a partly mineralized matrix is laid down in an incremental pattern; (b) increasing mineralization spreads out from the amelodentinal junction and is seen first in the region of the cusps; (c) the lingual enamel of incisors mineralizes before the buccal enamel, and (d) the surface zone of enamel in the later stages of development shows bands of mineralization parallel to the striae of Retzius. When advanced mineralization can be seen in microradiographs of ground sections, a considerable amount of organic matrix is retained in decalcified sections of contralateral teeth. The pattern of staining reactions does not exactly follow the pattern of mineralization, and it has been found that the loss of organic matrix in decalcified sections cannot be used as an accurate guide to the degree of mineralization of enamel.

7. The Distribution of Enamel Lamellae in the Longitudinal Plane.—*E. A. Marsland, Department of Dental Pathology, University of Birmingham.* The incidence and distribution of enamel lamellae were investigated in unerupted teeth. Ground sections, ground surfaces, and decalcified sections were examined and lamellae were shown to be present before eruption. To demonstrate the true organic nature of lamellae, transverse ground sections of both fixed and unfixed teeth were decalcified and the lamellae isolated as organic strands running from the enamel-dentine junction to the surface of the enamel. In the longitudinal plane, lamellae were seen to be leaf-like structures extending from the cementoenamel junction toward the incisal edge or occlusal surface. The coronal limit of a lamella was found to be irregular in form and to have no constant relationship to other structures of the enamel. Lamellae do not pass over onto the occlusal surface and rarely extend higher than the level of the dentine cusp. A shorter variety of lamella was seen more commonly; this type was limited in its distribution to the cervical third of the crown. The upper edge of a lamella was frequently seen to run obliquely from the enamel-dentine junction to a higher level on the outer surface of the enamel. This distribution accounts for forms of lamellae seen to extend only part of the way through the enamel in transverse sections.

8. Studies on the Mineralization Pattern of Human Enamel.—*J. H. Allan, Department of Oral Pathology, University of Sheffield.* The enamel of human deciduous teeth, covering a period of development from 26 weeks in utero to 18 months postnatal, was examined. The appearances of routine decalcified sections were correlated with the x-ray absorption and polarized light findings in ground sections prepared from the same teeth. The polarized light and x-ray studies show that crystalline mineral matter is deposited soon after the formation of the organic matrix. In the early stages of enamel development, the concentration of this mineral matter is greatest in the first formed matrix bordering the incisal portion of the amelodentinal junction. With increasing age, the mineral deposition then gradually advances peripherally and cervically through the whole enamel—the basic pattern of this advance being initially roughly parallel to the striae of Retzius, and finally roughly parallel

to the enamel surface. Decalcified sections of the same teeth show the development of an area of "soluble" matrix in the incisal enamel which, with increasing age, expands cervically along a front approximately at right angles to the enamel surface. Correlation of the x-ray absorption, polarized light, and decalcified section findings show that no close relationship exists between the area of "soluble" matrix and the basic mineralization pattern. These studies support the view that enamel mineralization starts at the amelodentinal junction and advances peripherally toward the enamel surface.

9. Some Histological Findings in Fissure Caries.—*E. B. Manley and E. A. Marsland. Department of Dental Pathology, University of Birmingham.* The occlusal fissures of molar teeth showing no signs of clinical cavitation were selected for microscopic examination. Ground surfaces, ground sections, and decalcified sections of each specimen were prepared. Beneath brown-stained fissures, early caries of the enamel was seen but examination of decalcified sections indicated only slight change in the organic matrix of the enamel at this stage. In deep unstained fissures of clinically caries-free teeth, early carious lesions were found at the bottom of the fissure. More advanced lesions at this site showed the typical cone-shaped area of carious or "altered" enamel (Hardwick and Manley, *Brit. D. J.* 92: 225, 1952) with its increased affinity for stains and greater resistance to acid action than the adjacent normal enamel. The zone of altered enamel was in advance of any bacterial invasion. Cavitation occurred toward the bottom of the fissure and appeared to be the result of invasion and removal of the altered enamel by microorganisms. Lamellae at the bottom of fissures were found to be infected along their entire length. Spread of infection from the lamellae both laterally along the amelodentinal junction and into the dentine appeared to occur only when altered enamel was present and had reached the dentinal surface.

10. The Early Lesion of Enamel Caries.—*A. I. Darling, Dental School, University of Bristol.* Examination of the translucent zone around the carious lesion of enamel in polarized light in air shows that this zone contains spaces caused by caries. Contrary to the hypothesis that this zone is hypercalcified, it shows in fact a very early stage of the carious attack. By the use of polarized light on specimens in various media and by microradiography, it can be shown that the spread of the carious lesion within the enamel is first along what we call the interprismatic substance then across the transverse striations and from these along the body of the prism. During this process, the prism cortex, the surface zone of the enamel and, to a less extent, the zones immediately beneath the striae of Retzius remain quite resistant to decalcification. The striae of Retzius themselves seem to aid the rapid spread of the lesion. The entry of caries can be shown to take place along the striae of Retzius. In the earliest lesions this can be shown by polarized light in air while in later stages it can be seen microradiographically.

11. The Differential Staining of 5-Micron Sections of Undecalcified Mature Human Dentine and Pulp.—*H. Allred, Turner Dental School, Manchester.* The detailed histologic examination of thin sections of undecalcified, mature human teeth, including pulpal tissue, was possible when teeth were embedded in acrylic prior to sectioning with a disc and subsequent milling between two stones. After etching and partial hydrolysis in hot 0.02 per cent acetic acid, the following differential staining occurred, using Pollak's trichrome stain: enamel, green; cementum, orange; pulp, faintly green and orange; dentine, clear differentiation of inter- and peritubular dentine. The latter had a fairly constant diameter (5 μ) and was differentiated into a central core (Tomes

process) a lightly stained zone, approximately 3 μ diameter, and a heavily stained periphery. The approximation of these peritubular zones toward the pulp at the expense of intertubular dentine was clearly shown. In regions adjacent to the predentine, and enamel, and in most of the root, peritubular and intertubular dentine were differentially stained orange-red, elsewhere peritubular zones stained blue-green. It is suggested that the odontoblasts produce the peritubular dentine; von Korff's fibers being responsible only for the intertubular material. The basic stain is possibly associated with greater calcification.

12. Some Effects of Vitamin A on the Growth and Development of Jaws and Teeth.—*May Mellanby, 5 East Heath Road, London, N.W. 3.* Lateral expansion of the mandible involves resorption of bone on the lingual side and deposition on the buccal. In vitamin A-deficient dogs, this remodelling process is disturbed, osteoclasts and osteoblasts being active in some positions where they are normally inactive, and vice versa. This results in a thicker mandible with a narrower internal dimension, the alveolar crest and lamina dura are also irregular and thickened. There is a crowding of teeth, some of which may have a pronounced lingual inclination, the roots are stunted and there is an excessive amount of cementlike substance in the apical foramen, constricting the nerves and blood vessels (Mellanby and King, *Brit. D. J.* 56: 538, 1934). Vitamin A administration tends to reverse these processes, osteoblastic and osteoclastic activity returning to the normal for the age of the animal. The bones, although disorganized, are well calcified if vitamin D is included in the diet, but, if this is deficient as well as vitamin A, these phenomena are accentuated.

13. The Effect of Acid Drinks on the Molar Teeth of Rats.—*P. J. Holloway, May Mellanby and R. J. C. Stewart (M.R.C. London).* Several American studies have shown that Coca-Cola and other soft drinks popular in the United States cause erosion of rat molar teeth when given to these animals to drink. The present study was made to see whether acid beverages and confections sold in this country have the same effect. Groups of rats were given the drinks as their sole fluid intake, 20 ml. per day for 5 days, and then killed. The molar teeth were examined for erosion, which was scored by the Restarski scale (Restarski, Gertner, and McCay, J. A. D. A. 32: 668, 1945). Several popular brands of fruit squashes and aerated drinks were tested, the former suitably diluted and the latter when most of the gas had effervesced. All of them caused some erosion, mainly of the lingual aspects of the lower molars. The fruit squashes, containing citric acid, were more potent than the aerated drinks, whose acid content was mainly phosphoric and lactic acids. No erosion resulted when distilled water or sugar solutions were given. The addition of sugar to citric or phosphoric acid solutions increased their erosive powers, as did saccharine also. Sodium fluoride, sodium oxalate, and sodium benzoate, the latter used as a preservative in fruit drinks, all served to reduce erosion.

14. The Development of the Gingival Epithelium in the Golden Hamster.—*W.D. McHugh, Department of Dental Surgery, University of Birmingham.* During maturation of the enamel in the golden hamster, the enamel organ is composed of the reduced ameloblasts and an irregular outer layer of stellate epithelial cells. As the erupting tooth nears the oral epithelium, numerous mitotic figures appear in the outermost layer of cells of the enamel organ. The cells in this layer are more densely packed and stain more heavily in contrast to the intermediate cells next to the ameloblasts. When the tip of the cusp pierces the oral epithelium, the active outer layer of the enamel organ fuses with the oral epithelium and the combined layer proliferates apically

down the tooth. The inner layer of the enamel organ, including the ameloblasts, degenerates and appears to form the secondary enamel cuticle. As this layer progressively degenerates, kerato-hyaline granules appear in the surface cells of the adjacent fused layer of outer cells of the enamel organ and oral epithelium. This fused layer now forms the gingival epithelium and its surface toward the enamel and against the degenerating cells gradually becomes keratinized. Thus, in the hamster, it is difficult to see how there can be any organic epithelial "attachment" between enamel cuticle and gingival epithelium as degenerating cells are interposed between them.

15. Vascularization of the Condylar Cartilage.—*H. J. J. Blackwood, Royal Dental Hospital, London.* About the twentieth week of intrauterine life, vascular canals or septa appear in the newly formed condylar cartilage of the mandible. At first only one or two canals are seen in each cartilage, but as growth proceeds they become more numerous so that by full term they may total 7 to 9 in each cartilage. During the first year of postnatal life, when growth of the condylar head is rapid, a proportionate increase in the number of septa is also noted but thereafter they tend to decrease in number and finally disappear toward the end of the second year of life. The canals contain blood vessels which enter from the medullary cavity of the condyle and course upward toward the articular surface of the cartilage. On reaching the transitional layer of the cartilage most of the vessels turn at right angles and ramify within this layer for a varying distance over the head of the condyle. Some vessels form short anastomotic loops with adjacent vessels while others appear to anastomose with vessels from adjacent canals. It is assumed that the function of these vascular canals is to aid nutrition and interstitial growth of the cartilage during this early period of development.

16. A Craniometric and Dental Investigation of 301 Romano-British Skulls and Jaws Circa 150 A.D.—*C. Cooke and T. C. Rowbotham, Turner Dental School, University of Manchester.* Out of a number of inhumations discovered near York in 1951, 301 were found with teeth and jaws. Cranial measurement revealed European characteristics in the cephalic, gnathic, nasal, and facial indices. This pattern was continued when the dental and palatal indices of the jaws were calculated. General development of the jaws was excellent with well-marked muscle attachments. There were few instances of overcrowding of the teeth and, in 92.8 per cent of the cases, occlusion was normal (Angle, Class I). In the young adult both overbite and overjet were approximately 1.5 mm. but, after the age of 25 to 30, attrition resulted in edge to edge occlusion of the incisors. Carious lesions were noted in 226 teeth out of 4,963 examined. All except 4 of the lesions were situated in posterior teeth, mainly on the proximal surfaces in the older age groups. Evidence of periodontal disease was practically limited to the aged skulls. However, in areas of dysfunction attributable to the presence of advanced carious lesions, heavy unilateral deposits of supragingival calculus were often noted, together with exposure of the roots by absorption of alveolar bone.

17. The Total Pattern of Innervation in the Oral Mucosa.—*A. D. Dixon, Department of Anatomy, University of Manchester.* Correlation of several aspects of oral innervation—the nature of nerve terminations, the plexuses from which they arise, and the types of nerve fibers found therein—enables one to formulate a concept of its total organization. Major nerve bundles derived from the trigeminal nerve form plexuses in the submucosa which are confined in depth where the submucous tissues are thin and compact. Myelinated

fibers which make up the greater part of the plexuses are joined by nonmyelinated autonomic fibers, mainly of sympathetic origin. As the myelinated fibers proceed to their final distribution they branch dichotomously and decrease in size. Each fiber has a complicated course so that localized areas of subepithelial tissue are innervated by nerves which approach from several directions. The mode of distribution of nerve plexuses is reflected in the mode of origin of nerve endings for frequently stem fibers giving rise to sensory endings approach from more than one direction. The impression of plexus topography obtained from stained tissue sections has been substantiated using a photographic method of reconstruction (Dixon and Howarth, *J. Anat. Lond.* 92: 162, 1958). Reconstructions support the view that localized areas have a multiple innervation and reveal great variation in the total innervation pattern.

18. The Action of Filtrates from Cultures of Clostridium Histolyticum on Decalcified Bone and Dentine.—*J. A. Weatherell and G. Hobbs, Biological Research Unit, University of Leeds.* A method was developed for titrating collagenase which seemed superior to methods previously employed. It was established by immunological methods that decalcified bone was a specific substrate for *Clostridium histolyticum* collagenase (β toxin), and that dyeing the substrate with chlorazol fast pink did not affect its specificity. This provided an easy means of detecting breakdown of the collagen. It was found that dentine, decalcified and dyed in the same way, was not specific toward collagenase, but was also attacked by *Clostridium histolyticum* γ toxin. Identical results were obtained by Evans and Prophet (*J. Gen. Microbiol.* 4: 360, 1950), using a similar dentine preparation. It was shown that sections consisting of exostosed and cortical bone from decalcified tibia of fluorosed rabbit were attacked by the β toxin and not by the γ toxin. The cortical bone was attacked more readily than the exostosed bone and, in general, it seemed that recently formed structures were more resistant to attack by collagenase than older bone.

19. Dentine Destruction by Caries.—*E. W. Bradford, Dental School, University of St. Andrews, Dundee.* The approach of a carious lesion to the dentine seems to produce two opposite effects in the tissue. Either the tubules become occluded or no reaction is apparent. The two reactions can be seen in the same area of one tooth and even within adjacent tubules. The distribution of the two may be haphazard, or show a definite orientation to the carious lesion. The contents of the occluded tubules are resistant to bacterial attack after decalcification by the carious process and may be recovered from the floor of a carious cavity. Conversely, the tubules in which no reaction is apparent become enlarged at the expense of the peritubular dentine and ultimately form the pathway for the bacterial invasion of the tissue in the late stages of the disease.

20. The Occurrence of Branching Bacteria in Root Canal Cultures.—*T. H. Melville and L. F. Gore, School of Dental Surgery, University of Liverpool.* Gram-positive branching bacteria have been found to occur fairly commonly in 5-7 day Robertson's meat medium but not in 24-48 hour glucose broth cultures from root canals. In the meat medium or on subsequent blood agar cultures, these organisms are usually of a rodlike "diptheroid" morphology. When examined, either stained or unstained, in situ, on the surface of quarter strength Difco brain-heart agar, they appear as typically mycelial colonies in young culture. Further investigations indicate that these organisms are fairly abundant in the normal oral flora but their significance in root canal cultures is unknown. In pure culture the majority of these bacteria appear to belong to a

group of facultatively anaerobic organisms with a viscous type of growth, capable of lowering the pH to between 4.2 to 5.0 in glucose or sucrose media; nearly all can hydrolyze starch and some are proteolytic to gelatine but not much closer study of their role in the oral cavity.

21. Inheritance of Geminated Composite Odontome in the Dog-Pilot Experiments.—*A.D. Hitchin and I. Morris, Dundee Dental School, Queen's College, University of St. Andrews.* In the course of line breeding of Lakeland terriers, it was observed that unilateral gemination of the maxillary first and second primary incisors followed by gemination of their secondary successors was occurring with comparative frequency. This suggested a genetic investigation. A bitch with this condition present on the left side, and whose mother and grandmother were also affected, was mated with a dog with the condition on the left side. The resulting litter of 4 puppies consisted of 2 normal dogs, one normal bitch and one bitch with bilateral gemination of the first and second maxillary incisors of both dentitions. When tnis mating was repeated, a litter consisting of 2 normal dogs, 2 normal bitches and one bitch with the condition on the left side resulted. The same sire was mated with a normal bitch from the strain in which the unilateral condition had been observed. This produced a litter of 6, consisting of 4 normal dogs, one normal bitch and one bitch with bilateral gemination of the upper first and second incisors of both dentitions. It seems clear that this type of geminated composite odontome in the dog is inherited and that more than one gene is involved. Further investigation into its genetics is required, and the possibility that the bilateral condition may be homozygous deserves consideration.

22. The Influence of Alloxan Injections on the Development of Dental Caries in the Rat.—*R. L. Hartles and F. E. Lawton, School of Dental Surgery, University of Liverpool.* Everett, Lal, and Phatak (*J. D. Res.* 28: 653, 1949) claimed that alloxan diabetes leads to an increase in caries in hamsters. Nichols and Shaw (*J. D. Res.* 36: 68, 1957) could not demonstrate any such increase in rats. In the present investigation 37 4-week-old rats were injected subcutaneously with alloxan (200 mg./Kg. body weight). All the animals are maintained habitually on a purified diet containing 67 per cent sucrose (Hartles, Lawton, and Slack, *Brit. J. Nutrit.* 10: 234, 1956). At 6 months of age there were 31 survivors. The injected animals had a mean number of 4.7 carious teeth compared with a mean of 2.3 for the controls. The corresponding values for mean caries score were 8.2 and 4.0. These differences are significant ($P<0.01$). The injected animals showed some of the signs of diabetes (glycosuria, polyuria, and increased water intake) but their blood-glucose values were not notably greater than they were in the controls.

23. Effect of Ingested Carbohydrates on the Concentrations of Amylase, Orthophosphates and Carbon Dioxide in Human Parotid Saliva.—*J. F. Bates, Turner Dental School, Manchester.* Possible adaptation of the parotid glands to ingested carbohydrates has been investigated. (1) A comparison of the composition of saliva stimulated by wax and barley sugar has been made, and the results show that differences in the salivary composition are due to differences in the rates of secretion. This suggests that reflex stimulation of the salivary glands results in the same composition of saliva irrespective of the type of stimulant. (2) Changes in the composition of the blood may cause changes in the composition of the saliva and in order to investigate this, experiments were conducted with the following results: (a) No change was found in the amylase concentration of wax-stimulated saliva in 5 subjects who

reduced their carbohydrate intake for a period of 4 weeks. (b) Infusions of phosphate buffers (plasma levels of up to 3.0 mM/ L.) cause a 7 per cent rise in the phosphorus of wax-stimulated saliva. Hydrocortisone infusions and glucose taken by mouth had no effect on phosphorus levels of wax-stimulated saliva. (c) Glucose ingestion caused no change in the carbon dioxide concentration of wax-stimulated saliva.

24. The Effect of the Secretions of Human Submaxillary and Parotid Glands on Some Bacteria.—*A. C. Kerr, Department of Dental Medicine, Guy's Hospital, London, and Doreen L. Wedderburn, Unilever Ltd., London Road, Isleworth, Middlesex.* Two types of antibacterial factor have been described in whole saliva (Bibby, Hine, and Clough, *J. A. D. A.* 25: 1290, 1938). These factors may have originated in the cells and microorganisms of saliva or may have been secreted by the salivary glands. The present investigation was made to see if submaxillary and parotid secretions had antibacterial activity. Secretions were collected by cannulae from the appropriate gland ducts of 7 volunteers. No epithelial cells or leukocytes were found nor could viable organisms be demonstrated when samples were tested by standard methods. Assays for antibacterial activity were carried out in well-plates against 7 microorganisms. Controls used were lysozyme (10 mg. per cent), Ringer's solution, and distilled water drawn through the cannulae prior to collection of secretions. In addition, the effect of 6 hours' contact with submaxillary and parotid secretions on *Lactobacillus casei* and *Micrococcus lysodeikticus* was measured by initial and final viable counts. Results obtained by both methods suggest that submaxillary and parotid glands secrete at least 2 types of antibacterial factor. One is a lysozyme-like factor which is not destroyed by heating to 75°C. for 7.5 minutes and the other is a heat labile factor active against 6 lysozyme-insensitive organisms.

25. The Effect of Different Concentrations of Glucose on the pH of Dental Plaques In Vivo.—*I. Kleinberg, Physiology Department, Medical School, King's College, Newcastle-upon-Tyne.* The effects of different glucose concentrations on plaque pHs of selected interproximal areas have been studied with modified types of antimony electrodes by (a) keeping the glucose concentration constant at different levels and (b) allowing the concentration to fall from various initial values. With (a), the steady state pH was found to be a function of glucose concentration, in that, at low concentrations the pH drop is submaximal, reaching a maximum at approximately 5 per cent and then showing some inhibition at 50 per cent; which agrees in general with preliminary in vitro studies on saliva-glucose mixtures. With (b), as the glucose concentration falls, the plaque pHs at first also decrease and then rise as the glucose concentration decreases further. If, during its decrease, the glucose concentration remains above a certain critical level for a long enough time, then the pH minimum can be reached. In addition, the longer the glucose concentration, during its fall, is above this critical level, the longer the plaque pH is at its minimum. From these and unpublished results, a general theory for the variable effect of the different carbohydrate foods (Jenkins and Kleinberg, *J D. Res.* 35: 964, 1956) on plaque pHs has emerged.

26. Studies on the Disintegration of Leukocytes in Saliva on Incubation.—*D. E. Wright, Department of Physiology. Medical School, Newcastle-upon-Tyne,* 1. Previous studies (Wright and Jenkins, *J. D. Res.* 32, 511, 1953) showed that the count of complete, undamaged leukocytes—referred to as

"intact" cells—in saliva specimens from caries-free subjects was approximately 4 times greater than that observed in caries-active samples. Other experiments (Wright and Jenkins, *J. D. Res.* 32: 733, 1953) demonstrated the constancy with which this relationship was obtained despite varied methods of collection of samples. One possible explanation of this observation is that there exists a slower rate of disintegration of leukocytes in the mouths of caries-free subjects. Five samples of wax-stimulated saliva, taken at weekly intervals, were obtained from 4 caries-free and 5 caries-active subjects. An aliquot was diluted with an equal volume of 0.5 per cent saline and was incubated at 37° C. with agitation. At zero time, and at 5- or 10-minute intervals, counts were made of the intact leukocytes present and were continued until all intact cells had disintegrated. The results showed that despite a greater total number of intact leukocytes in the caries-free samples, the rate of disintegration was more than twice as great as in the caries-active samples. Thus, differences in the rate of disintegration cannot explain the observation that the count of intact cells is greater in caries-free subjects.

27. The Development and Atrophy of Buccal Sebaceous Glands in Man.—*A. E. W. Miles, The London Hospital Dental School.* Counts of sebaceous glands in the cheek mucosa of living subjects and autopsy material led to the conclusion that there is an increase in the number of these glands with age (Miles, *Brit. D. J.* In press). Developing stages were therefore sought in serial sections of autopsy material treated with Flemming's solution which preserved the fats in paraffin sections and so facilitated the recognition of early stages. From observations on sections from a 4-year-old child it appears that buccal sebaceous glands develop as epithelial buds in which the central cells become distended with fat which is discharged through the epidermis via what becomes the definitive duct. In specimens from two males, aged 32 and 55 years, several similar sebaceous buds, regarded as developing stages, were found. In 3 specimens from subjects, aged 45, 55, and 82 years, small atypical glands were found which had a shrunken appearance, were composed mainly of cells of squamous type and invested with an unusually thick fibrous layer. Several were associated with isolated fragments of sebum in the overlying epidermis. These glands were regarded as atrophic.

28. Variations in the Cytochemistry of Mast Cells of the Rat.—*B. G. Radden, Department of Dental Pathology, London Hospital Medical College.* Mast cells are classically identified by the presence within their cytoplasm of metachromatic granules. An acidified solution of toluidine blue containing magnesium ions was found to be specific for these granules, but the intensity of the stain showed marked variations. Mast cells contain heparin (Jorpes, Heparin, ed. 2, 1946, London, Oxford Univ. Press) and the degree of metachromasia of heparin with toluidine blue is proportional to the number of sulphate radicles with which the heparin is esterified (Lison, *Arch. de Biol. Paris* 46: 599, 1935, and Jorpes, 1946). Heparin monosulfate is the only sulphate ester of heparin which is PAS positive (Jorpes and Gardell, *J. Biol. Chem.* 176: 267, 1948). In this investigation the presence of sulphate groups within the mast cells has been established by positive results with methylene blue extinction tests and 1 per cent alcian blue at pH 0.2. The majority of mast cells of adult tongue, lip, and cheek stained strongly with the toluidine blue solution, and are PAS negative. Most of the mast cells in adult cervical lymph nodes and all those of fetal lip and cheek stained weakly with toluidine blue, and are PAS positive—even after treatment with diastase or hyaluronidase. These results are interpreted as evidence of the variations of

the sulphation of the heparin in the mast cells. Riley's classification (*J. Path. Bact.* 65: 461, 1953) of two types of mast cell is considered an oversimplification and it is suggested that the variations in the staining reaction represent different stages in the maturation of the mast cells.

29. The Distribution of Alkaline Phosphomonoesterase in the Developing Human Tooth.—*A. R. ten Cate, Anatomy Department, London Hospital Medical College.* Undecalcified sections of human developing teeth, fixed in chilled acetone, 80 per cent alcohol and 10 per cent formalin were used in this investigation. Alkaline phosphatase was demonstrated with the Gomori cobalt method and with azo dye methods. Enzyme activity first occurs in the newly differentiated cells of the stellate reticulum. Its function here is considered to be associated with production of an acid mucopolysaccharide by the stellate reticulum. A gradient of enzyme activity was found to occur in the external enamel epithelium, stellate reticulum, and stratum intermedium, attaining a maximum where these tissues overlie developing cuspal areas. After staining with azo dyes, the ameloblasts and the odontoblasts exhibited no enzyme activity. Positive staining which can be elicited in the ameloblasts and the odontoblasts with the Gomori cobalt method can be discounted, as this is attributable to the close proximity of these cells to sites of rich enzyme activity, in the stratum intermedium and the sub-odontoblastic zone, respectively. However, absence of alkaline phosphatase from the formative cells of dentine and enamel does not conflict with current theories of hard tissue genesis.

30. The Effects of Calcium Hydroxide on the Unexposed Pulp.—*D. J. Stewart and Ivor R. H. Kramer, Departments of Children's Dentistry and Pathology, Institute of Dental Surgery, University of London.* Many workers have shown that calcium hydroxide ($Ca[OH]_2$) gives better results than zinc oxide (ZnO) and eugenol when applied to the accidentally exposed pulp or following vital pulpotomy. More recently, it has become common practice to apply $Ca(OH)_2$ to the dentine where there is no pulp exposure, in the hope that the material will promote secondary dentine formation. The purpose of the present investigation was to compare the effects on the pulp of $Ca(OH)_2$, and ZnO and eugenol, when used in cavities without pulp exposure. Recently erupted human premolars were used, the cavities being prepared as described by Kramer and McLean (*Brit. D. J.* 92: 255, 281, 311, 1952). Some were filled with ZnO and eugenol; in others a paste of $Ca(OH)_2$ in water was applied to the cavity floor, covered with a layer of tinfoil, and the remainder of the cavity filled with ZnO and eugenol. The teeth were extracted after intervals of 21-84 days; sections were prepared and the pulp response assessed as described in the paper previously cited. No significant differences were found between the 2 groups.

31. Observations on the Cariogenic Effect of Adding Cane Sugar Syrup to the Diets of Rats.—*T. G. H. Davies, Department of Children's Dentistry, London Hospital Dental School, London.* To determine whether dental caries can be produced in a pure strain of rat, not known to be caries susceptible, syrup was added to Rank's 41 Diet. Fifty rats, representing 3 generations, have been on the experimental diet for periods up to 11 months. Ten of these rats reared on a normal diet, and placed on the syrup diet when 6 months old, have shown no carious cavities over periods of up to 8 months. In young animals which had been reared and maintained on the experimental diet for 7 months, carious cavities were found. Gross caries was present in the

latter group after 11 months. No caries has been found in 20 control animals. The carious lesions occurred first in the mandibular first and second molars. The lower third and upper molars were affected after longer periods. The lesions ranged from minimal fissure caries, to cavitation, and destruction of the crown to gingival level. Histologic examination of the lesions shows the carious process, both in the enamel and dentine, to be comparable to that seen in human teeth.

32. Mesial Migration of the Teeth in Macaca Rhesus.—*J. H. Scott, Anatomy Department, Queen's University, Belfast.* The interpretation of the method of facial growth depends to a large extent on whether the teeth migrate forward through the jaws or whether they maintain their position after eruption. Although there are some significant differences in cranio-facial morphology, including the persistence until adult life of a sphenoidal synchondrosis (between the postsphenoid and presphenoid elements), the *Macaca rhesus* monkey is close enough to man in skull morphology to render it a useful subject for growth analysis. In the present investigation, jaws have been x-rayed and then sectioned and stained for histologic evidence of tooth migration at different ages. Evidence of migration has been found. It is furthermore suggested that the mechanism, producing the migration of the teeth, is not due to pressure exerted from behind by the erupting molars, but is brought about by surface deposition beneath the mucoperiosteum of the gum so that the process of migration is itself a consequence of alveolar bone growth.

33. The Reattachment of the Periodontal Tissues Following Trauma and Infection.—*F. E. Hopper, Sutherland Dental School, King's College, Newcastle-upon-Tyne, 1.* Artificial pockets were created on the palatal aspect of maxillary premolar teeth in 5 cats to a depth of approximately 7 mm. below the gingival margin. Chisels and fissure burs were used to ensure that bone was removed together with periodontal membrane and the surface of the root. No other treatment was given and healing was allowed to occur naturally. The animals were sacrificed at intervals of 3 to 6 months after operation. Sections of maxilla were cut with the teeth in situ and stained with hematoxylin and eosin. Contrary to previous findings, a substantial amount of connective tissue reattachment was observed in 4 out of five cases. New cementum had been deposited over the cut surface of the tooth and new periodontal fibers were embedded in it. New alveolar bone was being laid down and, where the contour of the root surface had been changed by the bur, in such a way as to restore the periodontal membrane to a thickness corresponding to that seen on the unoperated side of the tooth. Shallow periodontal pockets containing calculus and plaque were seen, with evidence of chronic inflammation beneath the crevicular epithelium. The reattachment had therefore occurred in spite of the presence of chronic infection in the immediate vicinity.

34. Further Observations on the Occurrence of "Protective Factors" in Unrefined Foods.—*G. N. Jenkins, Department of Physiology, Medical School, Newcastle-upon-Tyne, 1.* Previous work (*J. D. Res.* 34: 777, 1955) on the presence in unrefined carbohydrate foods of substances which reduce the solubilities of ground dental tissues and calcium phosphate in incubated saliva has been confirmed and extended. At least 3 "protective" substances are present in black treacle, namely, calcium ions, a buffer, and an unidentified substance. In golden syrup, the protective effects are smaller and can be accounted for almost entirely by the calcium content and buffering power. Certain naturally occurring organic phosphates (e.g., phytate and phosphoglyceric acid) have been found to have a "protective" effect and, as these or similar substances are

present in black treacle, they may contribute to its action. In similar experiments with white or brown flour or bread, or extracts made from them, it has been found that less calcium phosphate dissolves in the presence of the unrefined materials. These foods do not differ significantly in buffering power or calcium content and their effect is probably brought about by the organic phosphate (mostly phytate) which they contain. The effects are as readily observed in acetic acid buffer as in incubated saliva, so that interference with salivary acid production cannot be a significant factor.

35. Observations on the Optical and Electron Microscopy of Human Cementum.—*C. H. Tonge and E. H. Boult, King's College, Newcastle-upon-Tyne 1.* Recently extracted teeth from individuals of known history have been used in this investigation. Ground sections have been prepared from one half of each tooth. Optical and electron microscope replicas have been taken from the remaining half of each tooth. Some specimens have been embedded in Bakelite before replication. This method has been devised in order to produce flat replicas of the edge of the tooth and is of particular value in the examination of the primary cementum of the cervical part of the root. Progressive etching of the tooth surface has been carried out and replicas made after each etching. The negative optical replicas have been used to study the general arrangement and position of structures prior to the examination of similar areas using the electron microscope replicas. Collagen fibrils are demonstrable, by means of the electron microscope, in the matrix of the cementum where their orientation is irregular. The embedded fibers show collagen fibrils grouped in parallel bundles and in primary cementum they lie at right angles to the dentinocementum junction. The fibrils found in the cementum have been compared with teased preparations, as well as with replicas, taken from the tendo achillis.

36. Defective Colour Vision and Shade Matching in Dentistry.—*J. Farrell, Sutherland Dental School, King's College, Newcastle-upon-Tyne, 1.* Shade matching is important in dentistry because the appearance of dental restorations can be spoilt by the use of filling materials or teeth which do not match the colour of their natural surroundings. This report is concerned only with the effect on shade matching ability of defects in colour vision, which is part of a wider investigation which includes such problems as alterations in colour produced by chemical changes over a period of time, and the difficulties of shade matching by artificial light. The ability of 12 dental students with defective colour vision to match dental shades is being tested. They are asked to make matches from manufacturers' shade guides, matching a tooth from one guide with an identical one from another. Twelve matches have to be made; some are easy, but others offer a number of very close alternatives, only one of which is exactly right. The results obtained show that while the subjects with defective colour vision have difficulty in distinguishing small variations in the colour of pink artificial gumwork, they are able to match tooth shades as accurately and easily as subjects whose vision is normal.

37. Differential Growth of Deciduous Incisors.—*M. V. Stack, M.R.C. External Staff, Dental School, University of Bristol.* The ratio between crown height and labiolingual width of the mandibular central incisor has been found the same at the fifth month of foetal life as in the completed tooth (Moss and Applebaum, *J. D. Res.* 36: 644, 1957). Measurements on groups of 20 enamel-free developing incisor crowns confirm that shape is maintained from the completion of the tip until the curvature of the lingual surface reverses. The mean

height: width ratio for lower central incisors was 1.6 (upper, 1.4), and for lower lateral incisors 1.5 (upper, 1.3); standard deviations were 0.1. Heights of enamel-free upper central crowns were proportional to cube roots of their dry weights. Previous comparisons between weights of deciduous crowns and their times of growth had indicated a relationship K log W = log T, where T was the dental age and K, for central incisors, was 0.65 (upper) or 0.75 (lower). Relating ages to square roots of crown weights of all types of teeth allowed comparison of their growth rates, as rectilinear graphs in these terms, on a foetal age scale. The general formula preferred is $W^{½} = GT - A$, where G is a measure of growth rate. The growth rate of the upper central incisor, as calculated, exceeded that of the lateral by one fifth; those of the lower central and lateral incisors differed slightly.

CHICAGO SECTION

OF THE

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Programs for 1957-1958

Compiled by Sholom Pearlman, Secretary-Treasurer, Chicago Section

Five meetings of the Chicago Section were held during the year 1957 to 1958, Harold R. Englander presiding. The following 19 papers were presented.

October 24, 1957

Experimental Periodontal Disease in Dogs.
Nicholas R. Marfino and Frank M. Wentz, Loyola University School of Dentistry.

Casting Shrinkage of Cobalt-Chromium Alloys.
Richard Earnshaw, University of Manchester.

The Dentition of the Magdalenian Female From Cap-Blanc.
Virginia Carbonell and Albert Dahlberg, Walter G. Zoller Memorial Clinic.

Marginal Penetration of Various Materials by I^{131}.
Robert Going and Harold Dute, University of Illinois School of Dentistry and Hines VA Hospital.

November 21, 1957

Addition of Bacteriostatic Agents to Acrylic Resins.
Arthur N. Bhan and Roger C. Michelsen, University of Illinois, College of Medicine.

Electromyographic Analysis of Masticatory Efficiency.
Samuel Pruzansky and Martin Pesek, Cleft Palate Center, University of Illinois.
I. Qualitative analysis (presented by Dr. Pruzansky).
II. Quantitative analysis (presented by Dr. Pesek).

Chloride Levels in Parotid Saliva.
Harold R. Englander, Kirk Hoerman, and William J. Carter, Great Lakes Naval Training Center, and University of Kansas City School of Dentistry.

January 23, 1958

Microbial Decomposition of Enamel and Dentin in Vitro.
Arthur N. Bahn and Irwin Weinstein, University of Illinois, College of Medicine.

Electron Microscopic Study of Periodontal Exudate.
L. McMillan, D. Y. Burrill, and L. S. Fosdick, Northwestern University, School of Dentistry.

Naval Dental Research in Egypt.
M. M. Mazzarella, Dental Research Facility, U. S. Naval Training Center, Great Lakes.

Studies in Experimental Traumatism.
F. M. Wentz and H. Staffileno, Loyola University, School of Dentistry.

April 24, 1958

STRUCTURAL DEFECTS IN FILLING MATERIALS AS REVEALED BY RADIOISOTOPES.
Robert Going and J. A. Ralph, University of Illinois College of Dentistry and Hines Hospital.

DEVELOPMENT OF COLOR STANDARDS IN DENTAL MATERIALS.
W. T. Sweeney, Dental Research Section, National Bureau of Standards.

DIMENSIONAL ACCURACY OF ARTIFICIAL DENTURES: PROCESSING AND SERVICE.
George C. Paffenbarger, Dental Research Section, National Bureau of Standards.

MORPHOLOGY OF THE ORAL FUSOBACTERIA AS REVEALED BY THE ELECTRON MICROSCOPE.
Edward G. Hampp, A.D.A. Research Division, National Institute of Dental Research, National Institutes of Health.

May 22, 1958

THE SIGNIFICANCE OF THE MUCO-GINGIVAL JUNCTION.
W. A. B. Brown and Maury Massler, University of Illinois, College of Dentistry.

RADIOGRAPHIC STUDY OF THE RELATION OF THE MANDIBULAR ANGLE TO THE OPENING MOVEMENTS OF THE MANDIBLE.
B. J. Powers, J. R. Jarabak, and Harry Sicher, Loyola University, School of Dentistry.

THE EFFECT OF SUCROSE UPON THE ACID ETCHING OF HUMAN DENTAL ENAMEL SURFACES.
L. S. Fosdick, Lilia McMillan, and R. Quentin Blackwell, Northwestern University Dental School.

EFFECTS OF SALIVA ON DENTAL PLAQUE ACIDITY.
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*Supporting Associates have made contributions to the Association for support of the JOURNAL. These contributions are in multiples of one hundred dollars.